University of Alberta

Microbiological Studies of Polyacrylamide as a Flocculant Aid for Oil Sands Tailings

by

Melissa E. Haveroen

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ABSTRACT

Polyacrylamide was tested as a flocculation aid by Syncrude Canada Ltd. because of its effectiveness in flocculating tailings without elevating salt concentrations. Residual acrylamide at low concentrations in polyacrylamide formulations has raised environmental concerns, because acrylamide has been considered to be toxic. This study investigated the activities of oil sands microbial consortia in the presence of polyacrylamide or acrylamide under sulfate-reducing or methanogenic conditions in laboratory microcosms. Low concentrations of acrylamide were degraded under aerobic, sulfate-reducing, methanogenic, and simulated environmental conditions by several environmental microbial consortia. Acrylamide at higher concentrations stimulated methanogenesis after an acclimation period. When supplied as a nitrogen source, polyacrylamide significantly enhanced methanogenesis, but under sulfate-reducing conditions, no conclusions could be drawn about polyacrylamide use as a nitrogen source. Acrylamide does not persist under any of the conditions tested in this study, and so likely would not pose an environmental risk when present in oil sands tailings.

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LIST OF ABBREVIATIONS

AA	acrylic acid
AMD	acrylamide
cSt	centistokes
DNS	3,5-dinitrosalicylic acid
FID	flame ionization detector
FT	flotation tails
GC	gas chromatograph
HP	Hewlett Packard
HPLC	high performance liquid chromatography
MFT	mature fine tailings
MLSB	Mildred Lake Settling Basin
MW	molecular weight
NDIR	nondispersive infrared
OD	optical density
PAM	polyacrylamide
PDA	photodiode array
RP	reverse-phase
SRB	sulfate-reducing bacteria
STP	standard temperature and pressure
TC	total carbon
TEA	terminal electron acceptor
TIC	total inorganic carbon
TT	thickened tailings

1. Introduction

1.1. Oil sands operations

Organic polymers such as polyacrylamide (PAM) have been proposed to treat extraction tailings from oil sands operations in order to dispose of the fines fraction, to enhance heat recovery from extraction water, and to allow more rapid water recycle. However, the environmental stability of PAM and its residual contaminant acrylamide must be assessed before usage in oil sands projects beyond the tests conducted by Syncrude Canada Ltd.

Bitumen from oil sands accounted for 34% of Canada's domestic oil production in 2000, and will continue to be a significant source of Canada's hydrocarbon supplies (Syncrude 2000). Oil sands are found in four locations in Alberta, and of these, the Athabasca deposit is the largest, with an estimated volume of over 800 billion barrels of bitumen (FTFC 1995a), which is at least four times the size of the world's largest conventional oil field (Schramm et al. 2000). Production from conventional oil fields has been exploited historically, but as these reservoirs diminish, bitumen extracted from oil sands will become more important.

In general, oil sand ore is bitumen-impregnated sands containing a coarse sand fraction and a fines fraction of silt and clays, formation water, and bitumen (FTFC 1995a). The Athabasca oil sand deposit is composed of about 10 to 12% (wt.) bitumen dispersed in fine quartz sands, with an average grain diameters of 150 to 250 μm, and clays dominated by kaolinite and illite (Schramm et al. 2000). Commercially, successful recovery of the bitumen fraction is the goal of oil sands operations. Bitumen is a complex mixture of high molecular weight organic compounds such as hydrocarbons (FTFC 1995b). The bitumen in these sands is separated from the mineral fraction (sand, clay, and silt) by a thin water film, a unique property that allows for bitumen extraction from the oil sand using aqueous digestion methods (Schramm et al. 2000).

Currently, most bitumen is recovered from the oil sand at Syncrude Canada Ltd. by using the Clark Hot Water Extraction process. In this process, warm water with a process aid such as sodium hydroxide are added to oil sand and mechanically agitated to

form a slurry. The slurry is directed to settling vessels, where the bitumen is separated from sand and suspended fines by flotation (FTFC 1995b). The bitumen is then upgraded, producing a light sweet oil of lower viscosity (Syncrude Sweet Blend) that is pipelined to market for further refining (Syncrude 2000).

The material remaining following bitumen extraction is referred to as tailings which are hydraulically transported as an aqueous slurry to settling basins (FTFC 1995b). Extraction tailings slurries will have densities of about 1.4 to 1.5 t m^{-3}, comprised of water (45 to 60 wt. %), coarse sand (40 to 55% wt. %) (85% greater than 22 μm), fine silt and clays (15% less than 22 μm), and residual hydrocarbons (0.3 to 1 wt. %) (Syncrude 2000; FTFC 1995b). Syncrude Canada Ltd., a major oil sand processor, follows a "zero discharge" policy, meaning that no process waters or tailings material are released into the environment off its operation leases. The extraction tailings are placed into large settling basins from which process waters are recycled back to the plant following gravity settling and densification (MacKinnon 1989).

Once there, the fine fraction will settle to a solids content of greater than 30%, at which point they are called mature fine tails (MFT), and the clarified water layer (release water) is removed from the surface for reuse in the extraction process (FTFC 1995b). While sand and coarse tailings quickly settle, the fines fraction was estimated to take many years to fully de-water through settling and consolidation due to their small size and mineralogy (Eckert et al. 1996). During its first 25 y of Syncrude's operations, MFT have been accumulating at a rate of approximately 0.15 m^3 t^{-1} oil sand processed (M. MacKinnon, Syncrude Canada Ltd., personal communication, 2004). During the operation stage of development, continuous tailings addition compounds the sedimentation problem, because the fresh tailings stream disturbs the deposit. Eventually these soft tailings must be reclaimed. Although the release water is recycled, the remaining soft tailings must be reclaimed to meet the company's commitment to produce a stable, viable, self-sustaining habitat (FTFC 1995a). Two reclamation methods have been proposed: a "wet-landscape" approach, in which a water cap is placed over MFT to form lakes and wetlands, and a "dry landscape" reclamation option in which solidified tailings are reclaimed as a dry landscape (FTFC 1995b). The challenge facing Syncrude

and the other oil sands operators is how to produce such a reclaimable surface on which vegetation can succeed.

To increase the fine tailings sedimentation rate, chemical additives can be used. In one process based on coagulation, calcium in the form of gypsum ($CaSO_4 \cdot 2H_2O$) is added to form quick-settling stable aggregates, known as consolidated or composite tailings (Matthews et al. 2001). However, high gypsum application rates are required for effective coagulation, leading to elevated salinity concentrations in the tailings and release water (MacKinnon et al. 2001). High ionic strengths cause problems with the quality of release water for recycling (related to divalent cations and ionic strength) (MacKinnon et al. 2001) and with terrestrial reclamation of the resulting deposits (salinity, ionic composition) (Renault et al. 1998).

To avoid this problem, synthetic organic polymers, such as PAM, have been proposed as flocculation aids. Flocculation occurs when divalent cations form bridges between the anionic PAM and negatively-charged clays (Nadler and Letey 1989). Multiple charged groups on the PAM macromolecule adsorb many clay particles, thereby forming larger flocs that settle more rapidly out of suspension (Sworska et al. 2000). In addition, hydrogen bonding between clays and PAM contributes to adsorption of clay particles to PAM (Nabzar et al. 1986). PAM (75 to 300 g t^{-1} solids) is added to the flotation tailings stream, which originates directly from bitumen extraction. These flotation tails are lower density and have higher fines content than the primary extraction tailings. They will range from 4 to 24% solids, with the fines fraction generally greater than 50%. Mixing this tailings stream with PAM, followed by a rapid de-watering step in a thickener, produces a paste-like material referred to as thickened tailings (TT). Released water from the thickening process will contain a low solids content (less than 0.5%), and is available from the thickener rapidly enough to allow the potential for heat energy recovery. The resulting dense stream, produced from the underflow of the thickener (TT slurry), is pumped into storage pits, where further TT pore water is released over time. An overview of this process is shown in Figure 1.1. It is anticipated that large tailings ponds are not required for this process, because most tailings water is removed at the thickener, and the resulting TT deposit contains a higher fines density than conventional tailings (Syncrude 2000). The composition of the TT is 75 to 80%

solids (30 to 35% are fines less than 46 μm), with 0.2 to 0.4% bitumen (M. MacKinnon, Syncrude Canada Ltd., personal communication, 2004). The TT deposit is expected to allow for more efficient water recycle and faster access for terrestrial reclamation. The concern in this process is the concentrations of acrylamide present in TT resulting from PAM use, as well as the environmental fate of the PAM itself. A dosage of 25 g PAM m^{-3} treated tailings would results in acrylamide concentrations of 2.5 to 15 $\mu g\ L^{-1}$ in TT pore waters (Kindzierski 2001). Currently, Albian Sands Energy Inc. is the only oil sands company that produces TT on a large scale. Syncrude is carrying out pilot-scale experiments to evaluate the TT process, with emphasis on rapid water and heat recovery for the tailings for water reuse.

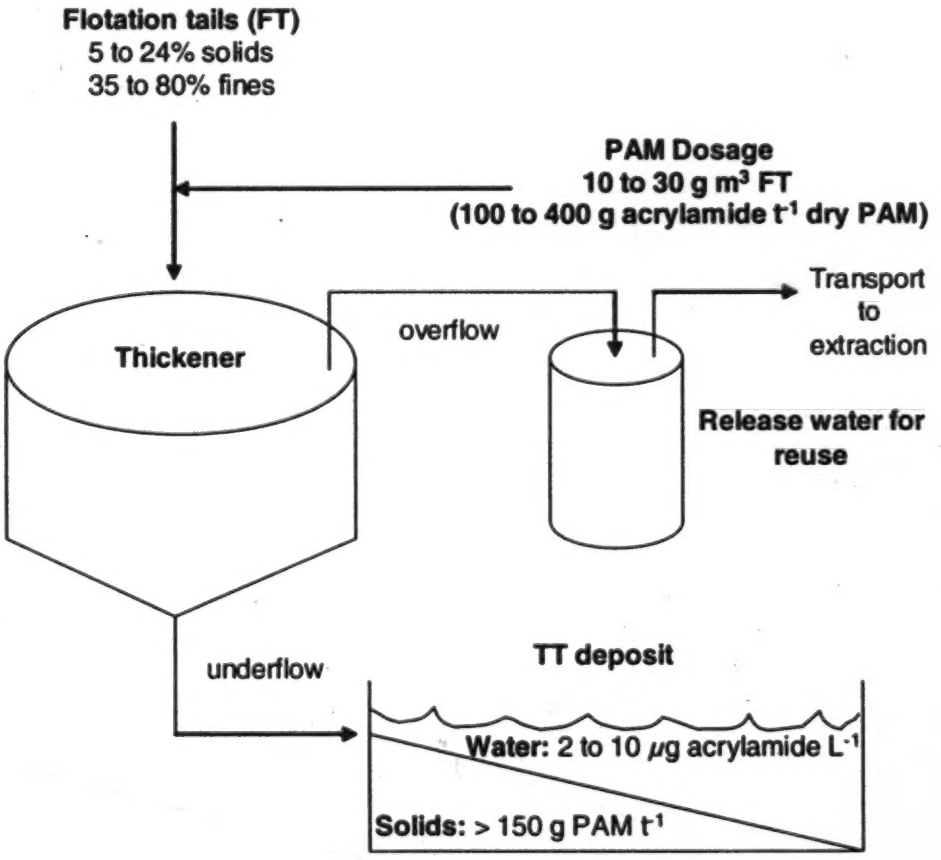

Figure 1.1. Schematic of the proposed TT process (shown by permission of Syncrude Canada Ltd.).

1.2. PAM

1.2.1. Properties of PAM

PAMs are high molecular weight synthetic polymers produced through copolymerization of acrylamide with alternate functional groups. The resulting polymers have various charges (nonionic, anionic, and cationic) and charge densities that provide enhanced versatility (Lipp and Kozakiewicz 1991). Anionic PAM, shown in Figure 1.2, has many industrial applications (European Chemicals Bureau 2002). It is produced by copolymerization of acrylamide and acrylic acid. Cationic PAMs which are more water-soluble are copolymers of acrylamide and quaternary esters or amines, whereas nonionic PAMs are copolymers of acrylamide. Depending on application, specific formulations can be made to optimize the PAM to specific uses. In the oil sand industry, most of the PAM used is anionic (Figure 1.1). Chain length determines polymer properties and usefulness, so PAM is classified according to weight-average molecular weight, as seen in Table 1.1. PAM is relatively water-miscible at all temperatures and pH values expected in tailings waters, although high molecular weight polymers may require longer for dissolution (Lipp and Kozakiewicz 1991).

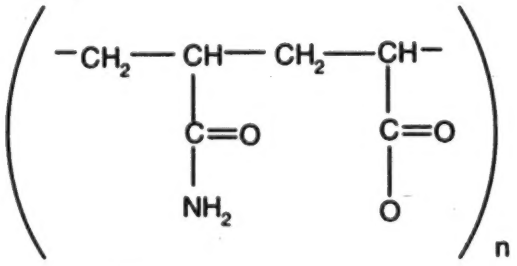

Figure 1.2. Representative structure of anionic PAM.

(n represents up to 10^3 subunits.)

Table 1.1. PAM classification based on molecular weight.

(adapted from Lipp and Kozakiewicz 1991)

Size classification	Weight-average molecular weight (M_w)
High	15×10^6
Low	2×10^5
Very low	2×10^3

During synthesis of the anionic PAM, incomplete polymerization leaves unreacted acrylamide in PAM formulations. Although most residual acrylamide is removed by chemical treatment, some remains as a contaminant in the finished product (Murgatroyd et al. 1996). Toxicity concerns surrounding residual acrylamide are discussed in Section 1.3.3. Manufacturers follow a 0.1% (w/w) guideline for the amount of acrylamide in PAM products (STOWA 1995), but most industrial PAMs have less than 0.05% residual monomer in the PAM, which is the maximum for potable water treatment facilities where allowable acrylamide concentrations are set at 0.5 $\mu g\ L^{-1}$ (U.S. EPA 1994). Recent studies have found that commercial PAMs considered for use in oil sands tailings flocculation have residual monomer contents from 140 to 460 $\mu g\ g^{-1}$ (0.014 to 0.046%) (Semple et al. 2001). Although release waters from polymer-treated tailings are not used as potable water sources, eventual reclamation of TT or seepage and runoff waters from TT deposits raise concerns over the concentrations of acrylamide present in the pore waters. Bologna et al. (1999) reported that low concentrations of acrylamide were detected in vegetables grown in PAM-treated soil. Thus, concern over availability of acrylamide in reclamation of TT has been raised, since the proposed PAM dosages are high enough that acrylamide in the pore and release waters would exceed the 0.5 $\mu g\ L^{-1}$ concentration established by the U.S. EPA (1999) unless *in situ* degradation processes occur.

1.2.2. Uses of PAM

Many industries, including water treatment, mineral processing, agriculture, and some of the oil sands processors, utilize PAM as a flocculant to enhance settling of

dispersed solids. As described in Section 1.1, divalent cations in solution form bridges between anionic PAM and negatively charged solids such as clay. Many charged groups are present on high molecular weight PAM, so many small particles are bound together into larger flocs which settle more rapidly out of solution than the original small particles, according to Stokes' Law (Mortimer 1991). Either cationic or anionic PAMs can be used as flocculants, depending on the predominant chemical charge of dispersed solids. Sewage sludge dewatering is often accomplished by cationic treatment, whereas anionic PAM is used for settling mineral solids such as gravel tailings, industrial effluents (Mortimer 1991), and flocculating oil sands tailings (Lord 2001, Jewel et al. 2002).

PAM has been used successfully for erosion control and improving soil structure since 1995 in the United States. Worldwide, over 400 000 ha have been treated with PAM to flocculate water-suspended particles (Sojka and Lentz 1997). One study found that PAM treatment at a concentration of 10 mg PAM L^{-1} reduced soil erosion by an average of 94% during a 3-y period (Entry and Sojka 2000). PAM has been shown to stabilize existing soil aggregate structure and prevent further aggregate decomposition by increasing soil cohesion, although it will not improve already-degraded soils (Sojka and Lentz 1997).

Anionic PAMs are used in a variety of industries including enhanced oil recovery operations, paper manufacturing, kimberlite tailings in diamond operations (Crocquet de Rosemond 2002), and food processing (Lipp and Kozakiewicz 1991; European Chemicals Bureau 2002). In oil field operations, PAM is used to modify drilling mud viscosity, provide structural integrity to drilled wells, and to control fluid loss from wells (Mortimer 1991). In enhanced oil recovery, high molecular weight anionic PAM is injected into oil wells to increase water viscosity and enhance oil displacement in a process known as polymer flooding (Lipp and Kozakiewicz 1991). In paper manufacturing, PAM addition strengthens interfiber bonds, thereby increasing paper strength (Lipp and Kozakiewicz 1991). In diamond operations in northern Canada, PAM (a mix of cationic and anionic) is used to treat the processed kimberlite tailings prior to disposal (Crocquet de Rosemond 2002). PAM has also been used in sugar

manufacturing, fruit and vegetable processing, and as a thickener in cosmetics (Lipp and Kozakiewicz 1991).

1.2.3. Toxicity of PAM

If reclamation of TT at oil sands operations is based on "wet landscapes" options, then aquatic organisms will be in direct contact with the deposit or waters released from the deposit. PAM is not toxic to aquatic organisms, bacteria or mammals because of its very high molecular weight. McCollister et al. (1965) determined that Lake Emerald Shiner fish tolerated 6000 mg L^{-1} of a nonionic PAM for 6 d with no observable ill effect. Yellow perch survived exposure to 100 mg L^{-1} for 90 d with no adverse effect, whereas fathead minnows, rainbow trout, and bluegills exposed to 1, 10, and 100 mg L^{-1} of an anionic PAM demonstrated no differences in feeding habits, behavior, or mortality. In treating tailings from diamond mining and processing, a combination of anionic and cationic PAM were applied, and possible chronic toxicity was evident through bioassays (Crcquet de Rosemond 2002).

Material safety data sheet information for various PAMs report toxic effects only at high concentrations. For example, the EC_{50} for *Daphnia* spp. is 212 mg L^{-1}, for algae is greater than 1000 mg L^{-1}, for the bacterium *Pseudomonas putida* is 892 mg L^{-1}, and the LC_{50} for freshwater fish is 357 mg L^{-1} (Ciba Specialty Chemicals Canada 1988). At high concentrations, the toxicity exhibited in fish and insects is attributed to viscosity of the solutions tested (European Chemicals Bureau 2002).

In mammals, few toxic effects have been reported for PAM. McCollister et al. (1965) performed toxicological studies on rats and dogs using anionic and nonionic PAMs, and found that no specific toxic effects were demonstrated at high ingestion dosages. No internal absorption of either the anionic or nonionic polymer was demonstrated through radiolabeled polymer studies (McCollister et al. 1965). Polyacrylate, the deaminated form of PAM, did not produce any adverse effects up to doses of 1000 mg kg^{-1} body weight (Hicks et al. 1989). When rats were injected intravenously or intraperitonealy with the polyacrylate solutions, a low molecular weight polyacrylate produced no toxic effects, whereas a high molecular weight polymer resulted in cardiovascular failure, haemorrhagic lesions, and general collapse (Hicks et

al. 1989). Such results indicate that the size of the molecule is important in causing toxic effects, and that large molecular weight polymers must bypass membranes to produce toxic effects, as seen in the toxicity with intravenous and intraperitoneal applications of polyacrylate (Hicks et al. 1989).

McCollister et al. (1965) concluded that nonionic and anionic PAMs may be safely used in applications where small doses of exposure occur, such as in the diet of humans or animals. Although its large molecular size makes PAM toxicity unlikely, acrylamide as a residual contaminant has focused attention on the degradation pathway of PAM (see Section 1.3.3).

1.2.4. Degradation of PAM

Once TT have been deposited, biodegradation could alter the added PAM. Three mechanisms of polymer degradation have been identified: scission of the main chain, depolymerization to the monomer or lower molecular weight fragments, and loss of ionic functionality (Gurkaynak et al. 1996). Unfortunately, because of the name "polyacrylamide", people wrongly think that depolymerization of this macromolecule will result in the release of acrylamide. This is extremely unlikely because, for acrylamide to form, the backbone of carbon-carbon bonds in the polymer must be broken in very specific locations and a double bond must be introduced between two of the carbon atoms of the resulting released fragment. Since post-deposition release of acrylamide from PAM in the TT is unlikely, the only source of acrylamide in the resulting PAM-treated TT would be residual acrylamide remaining from the PAM manufacturing process.

1.2.4.1. Chemical and physical degradation of PAM

In terms of PAM degradation, chemical methods such as ozonation (Kay-Shoemake et al. 1998a; Soponkanaporn and Gehr 1989; Suzuki et al. 1978) and chlorination (Aizawa et al. 1990; Soponkanaporn and Gehr 1989) effectively reduce the size of high molecular weight PAMs, though not to the monomer acrylamide. Chlorination may result in organic halide production from a reaction between PAM and

chlorine (Aizawa et al. 1990). Neither of these methods is relevant to tailings treatment as is used in TT, because PAM should remain bound in the solids phase.

During clarification processes using PAM, physical degradation by shear forces such as stirring, mechanical pumping, or movement through porous medium is a concern (Chmelir et al. 1980; STOWA 1995; Tolstikh et al. 1992). However, following thickening, TT are pumped to a remote site for deposition. Such movement could cause fragmentation into smaller oligomers that may be more accessible to microbial attack (Suzuki et al. 1978) but this does not appear to be the case, because chain length reduction by chemical or physical means was shown to increase biodegradation only marginally (Kay-Shoemake et al. 1998a; Soponkanaporn and Gehr 1989; Suzuki et al. 1978). El-Mamouni et al. (2002) subjected a nonionic ^{14}C-labeled PAM to UV irradiation, and found that 79% of the polymer was lyzed into fragments less than 1 kD after 72 h of treatment As predicted, these processes have not been shown to release the toxic acrylamide monomer (Soponkanaporn and Gehr 1989). Two questionable studies by Smith et al. (1996, 1997) reported that under laboratory and environmental conditions of light and temperature, depolymerization released acrylamide from PAM. At a constant temperature of 37°C, PAM was suggested to undergo physical changes leading to random breaks in the carbon backbone, thereby liberating acrylamide (Smith et al. 1996). However, this observation may have been due to residual acrylamide present in the original formulation, and no supporting evidence of this breakdown has been reported since. Once deposited, the TT will remain relatively stationary and physical PAM degradation is unlikely.

1.2.4.2. Biodegradation of PAM

Early biodegradation work involving PAM was focused on maintaining PAM stability, but limited microbiological study was carried out. For example, observed viscosity reductions in PAM solutions were attributed to biodegradation, because the addition of antimicrobial agents inhibited viscosity loss (Chmelir et al. 1980). However, this viscosity loss could not be directly attributed to microbial degradation of the main carbon backbone, because abiotic disruption of intermolecular hydrogen bonds (photolysis) could account for this observation (Chmelir et al. 1980). With respect to

remediation, stimulated microbial growth in PAM-treated soils suggested that PAM may be metabolized by soil microorganisms (Azazy et al. 1988; Zohdy et al. 1983; Grula and Huang 1981). Conversely, a toxicity effect was suggested by Steinberger and West (1991) who reported significantly lower nematode population densities in agricultural soil treated with PAM, and postulated that PAM reduced microflora on which nematodes subsist. Although toxicity is possible, PAM has been shown to be a sole nitrogen source for aerobic (Grula et al. 1994; Kay-Shoemake et al. 1998a,b; Nakamiya and Kinoshita 1995) and facultative anaerobic microorganisms (Nakamiya and Kinoshita 1995), and its amide group enhances growth of sulfate-reducing bacteria (SRB) (Grula et al. 1994).

In general, high molecular weight synthetic polymers with a carbon backbone demonstrate limited biodegradation unless the molecular weight is reduced to below 10^3 (Swift 1994), likely due to both high molecular weight and limited solubility (Iyer et al. 2000; Stahl et al. 2000). With an average molecular size of 0.2 μm, high molecular weight PAMs cannot enter microbial cells (Kay-Shoemake et al. 1998b). Thus, microbial attack on either the amide group or the carbon backbone likely involves extracellular enzymes. However, biodegradation is not enhanced by physical breakdown into smaller oligomers (Kay-Shoemake et al. 1998a; Soponkanaporn and Gehr 1989; Suzuki et al. 1978), as discussed in Section 1.2.4.1.

The PAM biodegradation pathway has not been fully characterized under either aerobic or anaerobic conditions. Acrylamide is would not be a product of PAM biodegradation; instead, propionamide ($CH_3CH_2CONH_2$) is a proposed product, which will likely be metabolized to propionate, and finally to CO_2 and ammonium (Wallace and Wallace 1995). Ammonium removal and use has been demonstrated (Kay-Shoemake et al. 1998a,b), leaving polyacrylate, which can be utilized as a carbon source by soil bacteria such as *Arthrobacter* spp. (Hayashi et al. 1993). However, the biodegradation products have not been identified and reported to date.

Certain aerobic microorganisms are able to use PAM as a nitrogen source. Extracellular amidases are believed to be responsible for PAM deamination, because extracellular specific activity for PAM was found to be higher than intracellular specific activity (Kay-Shoemake et al. 1998b). Mixed cultures enriched from soil (Kay-Shoemake et al. 1998a,b) and sewage sludge (Soponkanaporn and Gehr 1989), as well as

pure cultures of *Azomonas macrocytogenes* (Nakamiya and Kinoshita 1995) and *Pseudomonas* spp. (Grula et al. 1981, 1994) are capable of using PAM as a sole nitrogen source.

Microbial growth on PAM as a sole carbon source is limited. Neither pure cultures of *Pseudomonas* spp. (Grula et al. 1994) nor a PAM-enriched soil mixed culture (Kay-Shoemake et al. 1998a,b) could use a high molecular weight PAM as a sole carbon source. Mineralization of ^{14}C-labeled PAMs was reported to be less than 0.05% by *Pseudomonas* spp. (Grula et al. 1994) and less than 2% by an activated sludge culture (Schumann and Kunst 1991). A mixed culture demonstrated an ability to remove the cationic pendant group and ammonium from a cationic PAM, leaving the main chain intact (Chang et al. 2001). One study reported up to 85% degradation in 10 d (Yagaforova et al. 1999), but inadequate information regarding the residual monomer content in the PAM leads to difficulty interpreting these results. Nakamiya and Kinoshita (1995) reported growth of *Azomonas macrocytogenes* on PAM as sole carbon source, although no description of treatment to remove residual acrylamide from the laboratory-synthesized polymer was given. Soponkanaporn and Gehr (1989) also reported an activated sludge mixed culture capable of mineralizing PAM to CO_2, although their conclusions were based solely on disappearance of a high molecular weight polymer peak in size exclusion chromatography experiments, with no supporting CO_2 analysis. So far, the evidence verifying that microorganisms can use PAM as a significant carbon source is inconclusive.

The lignin-degrading fungus *Phanerochaete chrysosporium* has been shown to solubilize, mineralize, and incorporate PAM using extracellular enzymes, albeit at a slow rate (Stahl et al. 2000; Sutherland et al. 1997). A synergistic relationship between this fungus and soil microorganisms was also demonstrated, as PAM previously solubilized by the fungus was more readily mineralized than intact polymer, although only 0.70% was mineralized in 30 d (Stahl et al. 2000). A proposed biodegradation pathway using an extracellular enzyme by *Azotobacter beijerinckii* was proposed by Nakamiya et al. (1997), in which hydroquinone peroxidase reaction products abstract a hydrogen radical from the carbon chain, thereby splitting the chain and forming a double bond and a methyl group in both termini. A high molecular weight PAM (2×10^6) was degraded

into products less than 1000 MW, which were found to be units of three and five acrylamide monomers, as shown in Figure 1.2. No monomeric acrylamide was reported.

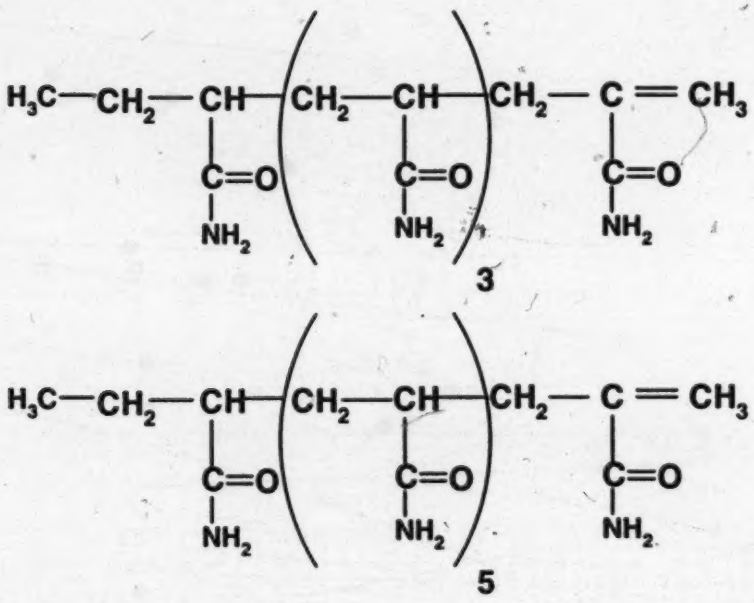

Figure 1.3. Chemical structures of PAM degradation products by hydroquinone peroxidase of *A. beijerinckii* (Nakamiya et al. 1997).

Very little research on biodegradation of PAM under anaerobic conditions has been reported. Schumann and Kunst (1991) reported no significant mineralization of a ^{14}C-labeled PAM by a sludge culture under anaerobic conditions. Chang et al. (2001) found that the carbon chain of a cationic PAM was not cleaved, although the amide moiety was hydrolyzed. Nakamiya and Kinoshita (1995) isolated the facultative anaerobe *Enterobacter agglomerans* reported to be capable of growth on the carbon backbone of PAM as a sole carbon source, although no ^{14}C-PAM analysis was done to support this claim. SRB native to an oil field were stimulated by high molecular weight PAM in an oil field, because PAM was found to increase growth of SRB, whereas

polyacrylic acid, lacking an amide group, caused no increase (Grula et al. 1994). Thus, PAM was believed to serve as a nitrogen source, but no amidase assays were done to verify this hypothesis. Although PAM is not believed to be used as a carbon source by SRB, the authors postulated that some type of metabolic reaction unique to SRB caused polymer degradation, because viscosity was decreased by SRB but not by other anaerobes. Because viscosity was reduced under respiratory conditions, such a reaction may be analogous to chemical degradation observed in the presence of oxygen (Thomas 1964). Recent laboratory studies have shown that anaerobic microbial activity occurs in MFT and composite tailings (Fedorak et al. 2002, 2003) and the addition of various substrates can stimulate microbial activity (Holowenko et al. 2001, Fedorak et al. 2002). The addition of PAM to tailings to form TT likely adds a useable source of nitrogen for microorganisms in the tailings and may add a source of biodegradable carbon. Thus, further research is required to elucidate if SRB and methanogens in tailings deposits will be affected by PAM application, or if floc destabilization will occur.

1.3. Acrylamide

1.3.1. Properties

Acrylamide (2-propenamide) is an unsaturated amide, shown in Figure 1.4. Its amide group is readily hydrolyzed chemically, yielding acrylic acid and producing free ammonium (Habermann 1991). One significant chemical property of acrylamide is its very high water solubility of 2155 g L^{-1} at 30°C (Habermann 1991), giving the potential for great mobility in aqueous environments (Kindzierski 2001). Acrylamide is produced chemically by acrylonitrile hydration, but enzymatic conversion of acrylonitrile to acrylamide has been explored as an economical alternative (Habermann 1991). Acrylamide readily copolymerizes with acrylates such as acrylic acid to form PAMs (Habermann 1991).

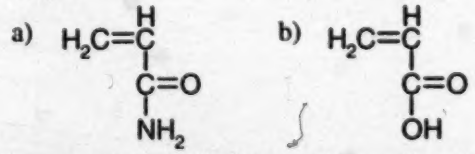

Figure 1.4. Structures of a) acrylamide and b) acrylic acid.

1.3.2. Uses of acrylamide

Industrially, acrylamide is used as a grouting agent for repair of sewer lines and manholes (European Chemicals Bureau 2002). For this application, acrylamide is polymerized at remote locations, forming an impervious layer to increase the strength of the surrounding medium or to reroute water (European Chemicals Bureau 2002). A small amount is used for research-grade PAM gels, but 95 to 100% of acrylamide is used in PAM or copolymer manufacture for industrial or commercial use (European Chemicals Bureau 2002).

1.3.3. Toxicity of acrylamide

Acrylamide has limited toxicity towards microorganisms, as indicated by the high LC_{50} concentrations of 13.5 g L^{-1} and 20.0 g L^{-1} for *Vibrio fisheri* and *Escherichia coli* (STOWA 1995). Although microorganisms may require lengthy acclimation periods, several genera of microorganisms have demonstrated biodegradation of various concentrations of acrylamide, as discussed in Section 1.3.6. However, acrylamide has also been reported to inhibit microbial growth (Clarke 1980). In mammals, acrylamide binds to sulfhydryl groups of glutathione-S-transferase, thereby inactivating the enzyme (Shanker et al. 1990). In microbial systems, the electrophilic vinyl group in acrylamide or its metabolites likely acts similarly, binding the sulfhydryl sites on enzymes such as amidases (Clarke 1980; Shanker et al. 1990). Sulfhydryl groups can be modified by vinyl compounds in the pH range 6 to 7.5, forming carboxyl side chains by hydrolysis of sulfur-alkylated proteins (Cavins and Friedman 1968).

Acrylamide is considered to be moderately toxic to aquatic organisms, with reported LC_{50} values of greater than 100 mg L^{-1} for various fish species and other aquatic organisms (Krautter et al. 1986; STOWA 1995; Kindzierski 2001). Krautter et al. (1986) exposed aquatic invertebrates and fish to various concentrations of acrylamide, and reported that daphnids and midge larvae demonstrated 24-h LC_{50} values of 230 and 570 mg L^{-1}, whereas rainbow trout, fathead minnows, and blue gills had 24-h LC_{50} values of 370, 320, and 260 mg L^{-1}, respectively. Krautter et al. (1986) concluded that aquatic

organisms would suffer no significant mortality when intermittently exposed to parts per billion concentrations ($\mu g\ L^{-1}$) of acrylamide. Conversely, Brown et al. (1980b) reported considerable mortality in aquatic invertebrates exposed to a 50 $\mu g\ L^{-1}$ concentration of acrylamide, but uncontrolled experimental parameters such as food availability and predatorial relationships cast doubt on their conclusions.

Acrylamide has been shown to cause neurotoxic, genotoxic, carcinogenic, developmental, and reproductive effects in mammals (Dearfield et al. 1988). When inhaled or ingested, acrylamide causes reversible neurological symptoms such as hallucinations, numbness, and ataxia (McCollister et al. 1964; U.S. EPA 1994). Acrylamide binds to DNA, causing heritable genetic damage and carcinogenic effects, thereby earning it a "probable human carcinogen" classification in the United States (U.S. EPA 1994). Fertility was markedly reduced in mammals by acrylamide, and developmental effects were observed in neonatal mice at levels nontoxic to the dam (Dearfield et al. 1988). Thus, acrylamide has been considered to be a highly toxic chemical, so low allowable acrylamide intake levels for water have been set by the U.S. EPA at 0.2 $\mu g\ kg^{-1}$ body weight d^{-1} (U.S. EPA 1994). Dosages of PAM in TT are estimated to produce acrylamide concentrations of 15 to 50 $\mu g\ L^{-1}$.

1.3.4. Acrylamide in foods

Recently, several research groups have reported that acrylamide formation during heating of foodstuffs is an important source of acrylamide exposure in humans (Mottram et al. 2002, Stadler et al. 2002, Tareke et al. 2002). When reducing sugars (such as glucose) and amino acids (primarily asparagine) are heated at temperatures above 100°C, acrylamide is formed via the Maillard reaction and Strecker degradation (Mottram et al. 2002, Stadler et al. 2002). Foods containing high concentrations of asparagine are particularly sensitive to acrylamide formation. Plant-based foods such as potatoes are quite susceptible to this reaction, because most contain high concentrations of asparagine, although acrylamide formation in meats was detected (Tareke et al. 2002). The predominant amino acid in potatoes is asparagine, at a concentration of 940 $mg\ kg^{-1}$, which is 90% of the total amino acid content (Mottram et al. 2002). Increased time and temperature of cooking was found to increase the concentration of acrylamide formed in

foods (Mottram et al. 2002, Stadler et al. 2002). Concentrations of acrylamide in some common foods are given in Table 1.2. Svensson et al. (2003) estimate dietary intake of acrylamide to be 31 $\mu g\ d^{-1}$, but these researchers caution that there is variability in acrylamide concentrations within food groups which make estimation difficult. Although acrylamide is considered a carcinogen (Dearfield et al. 1988), Mucci et al. (2003) failed to observe any association between acrylamide intake from food and increased rates of cancer of the large bowel, kidney or bladder. Thus, more research is required to fully elucidate the relationship between acrylamide and carcinogenicity.

Table 1.2. Concentrations of acrylamide in commonly-consumed food products.

Food product	Acrylamide ($\mu g\ kg^{-1}$)	Reference
French fries	300 to 1100	Svensson et al. (2003)
	300 to 750	Tareke et al. (2002)
	310 to 350	Ahn et al. (2002)
French fries (overcooked)	12 800	Ahn et al. (2002)
Crispbread	200 to 1700	Tareke et al. (2002)
	1340 to 4000	Ahn et al. (2002)
	Less than 30 to 1900	Svensson et al. (2003)
Popcorn	365 to 715	Svensson et al. (2003)
Coffee (medium roast)	25	Svensson et al. (2003)
Bread	Less than 30 to 160	Svensson et al. (2003)
	13 to 49	Tareke et al. (2002)
Breakfast cereal	Less than 30 to 1400	Svensson et al. (2003)
Hamburger	14 to 23	Tareke et al. (2002)

1.3.5. Acrylamide in the environment

In England, Croll et al. (1974) analyzed effluents from industries using acrylamide or PAM, and found that acrylamide was present in concentrations ranging from 0.47 $\mu g\ L^{-1}$ in treated paper mill effluent to 42 $\mu g\ L^{-1}$ in coal tailings lagoons. Industries using acrylamide or PAM in processes are sources of acrylamide pollution,

although dilution during recycling or in receiving waters will reduce the concentration to within safe limits for potable water supplies.

Acrylamide is very mobile in the environment, as its neutral charge and hydrophilic nature combined with its high water solubility enable it to remain associated with the water phase. Mobility is influenced by soil type, and acrylamide migrates through sandy soils more readily than through silt (Lande et al. 1979). Adsorption to sludges and sediments does not occur, although residual acrylamide present in PAM flocculants may become entrained in flocculated sediments (Brown et al. 1980a). Thus, acrylamide has the potential of leaching into groundwater or surface water supplies. However, modification of acrylamide through reduction of the carbon-carbon double bond or by removal of the nitrogen atom eliminates neurotoxic effects (Smith and Oehme 1991); so degradation reduces the toxicity of acrylamide.

1.3.6. Degradation and removal of acrylamide

1.3.6.1. Chemical and physical removal of acrylamide

The high water solubility of acrylamide makes physical methods ineffective for removing acrylamide from contaminated water. Croll et al. (1974) investigated the effect of water treatment processes such as settling and filtering, and found that nearly all the acrylamide was recovered from the resulting water. Laboratory experiments in which the effectiveness of coagulation and sand filtration for acrylamide removal were investigated resulted in 95% and 93% recoveries of acrylamide respectively from the aqueous phase (Croll et al. 1974). Activated carbon was ineffective at removing acrylamide as well (Croll et al. 1974). Thus, physical removal methods are not options for acrylamide removal.

Chemical reagents such as sodium sulfite, bisulfite (Habermann 1991) or $KMnO_4$ (Croll et al. 1974) can be used to scavenge acrylamide monomer, but there are no published reports of practical application of this method. Chlorination under acidic conditions removes considerable quantities of acrylamide (Habermann 1991), although a pH of 1.0 was necessary to remove 100% of a 6 $\mu g\ L^{-1}$ acrylamide solution (Croll et al. 1974). At neutrality, chlorination resulted in no reduction of acrylamide levels (Croll et

al. 1974). In addition, toxic organic halides such as $CHCl_3$ and 2,3-dichloropropionic acid are byproducts of the reaction between chlorine and acrylamide, making chlorination a non-viable option for acrylamide removal (Aizawa et al. 1990).

1.3.6.2. Biodegradation of acrylamide

Amide-containing compounds are common targets for microbial enzymes, and are preferred if they easily enter the cell and readily hydrolyze to produce ammonium (Clarke 1980). Acrylamide is a small soluble substrate containing an amide moiety, and so is a likely candidate for biodegradation. Microorganisms have been found to deaminate acrylamide, producing acrylic acid and liberating ammonium, as seen in Figure 1.5. Lande et al. (1979) found no acrylic acid as a metabolite as expected during acrylamide biodegradation, and concluded that acrylic acid must be rapidly metabolized.

Acrylic acid undergoes further metabolism to acetic acid by *Alcaligenes denitrificans* (Andreoni et al. 1990), *Geotrichum* sp. and *Trichoderma* sp. (Dave et al. 1996); and β-hydroxypropionic acid by *Byssochlamys* sp. (Takamirzawa et al. 1993), *Alcaligenes denitrificans* (Andreoni et al. 1990), *Alcaligenes faecalis* (Ansede et al. 1999), and *Geotrichum* sp. and *Trichoderma* sp. (Dave et al. 1996). Qu and Bhattacharya (1996) found that acrylic acid is biodegradable under anaerobic conditions to acetate and propionate. Although acrylic acid is present as a contaminant in copolymers of acrylamide and acrylic acid at concentrations of 350 to 3570 $\mu g\ g^{-1}$ (Semple et al. 2001), it is not expected to accumulate as a result of either acrylamide degradation or as a contaminant in PAM itself.

Ammonia (or ammonium) is a common nitrogen source for many microorganisms (Brown 1980). The pKa of ammonium dissociation is 9.25, so at neutral pH most is present as ammonium (NH_4^+), whereas at more alkaline pH in the environment, ammonia (NH_3) will predominate (Brown 1980). Ammonium is easily metabolized, and so is not expected to accumulate following acrylamide hydrolysis, as seen in Figure 1.5. Numerous researchers have reported ammonium production from acrylamide biodegradation (Kumar and Kumar 1998; Nawaz et al. 1998; Shanker et al. 1990; Wang and Lee 2001). Abdelmagid and Tabatabai (1982) found that ammonium

can be oxidized to nitrite and nitrate aerobically, but accumulates under waterlogged conditions.

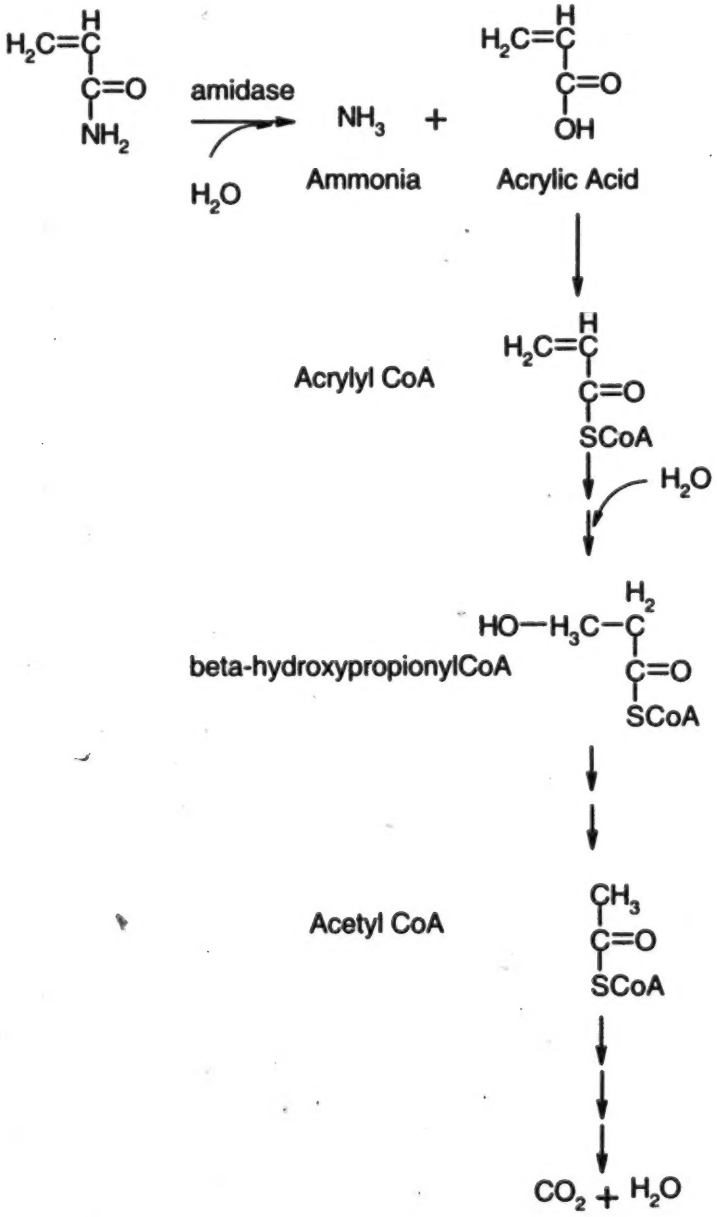

Figure 1.5. Biodegradation pathway of acrylamide (adapted from Shanker et al. 1990).

Amidohydrolases, commonly known as amidases, hydrolyze the carbon-nitrogen portion of amide bonds, producing the corresponding carboxylic acids and ammonium (Clarke 1980). Microbial amidases are widely produced intracellularly and extracellularly, and some microorganisms can produce more than one amidase capable of hydrolyzing a specific amide, but the enzymes are controlled by different regulation systems (Clarke 1980). Numerous microbial amidases with activity for acrylamide have been identified, as seen in Table 1.3. Sulfhydryl groups are important in many amidases, either at the active site or in tertiary structure (Ciskanik et al. 1995; Nawaz et al. 1996, 1998).

Although numerous amidases have been purified and characterized, the specific activities of most amidases towards acrylamide and other unsaturated amides are low compared to aliphatic amides (Ciskanik 1995). Acrylamide has been found to induce amidase activity in some microbial systems (Ciskanik 1995), but has been reported to cause poor induction in others (Hynes and Pateman 1970). Sulfhydryl proteins, including amidases, can be inhibited or activated by acrylamide.

Although amidases with activity against acrylamide have been identified from many aerobic microorganisms, no amidases from anaerobic microorganisms have been detected. However, because there does not appear to be an oxygen requirement for amidase activity, acrylamide deamination may occur under anaerobic conditions.

1.3.6.3. Biodegradation of acrylamide under aerobic conditions

Acrylamide is readily biodegradable to acrylic acid and ammonium under aerobic conditions (Abdelmagid and Tabatabai 1982; Lande et al. 1979; Kumar and Kumar 1998; Nawaz et al. 1994b, 1996, 1998; Shanker et al. 1990; Wang and Lee 2001). Biodegradation has been demonstrated under laboratory conditions numerous times, and although few field studies have been done, the resulting data from the completed studies discussed below indicate that acrylamide is degraded in soil and water, and therefore does not accumulate.

Table 1.3. Microbial amidases capable of acrylamide hydrolysis.

Organism	Activity (μmol NH_3 min^{-1} mg $protein^{-1}$)	Optimum		Size (kDa)	Inhibitors	Reference
		Temperature (°C)	pH			
Aspergillus nidulans	NS[a]	NS	NS	NS	NS	Hynes and Pateman (1970)
Arthrobacter sp. J-1	NS	55	7.0	300	Ag^+, Cu^{2+}, Hg^{2+}	Asano et al. (1982)
Brevibacterium sp. R312	10^{-1} [b]	NS	NS	NS	NS	Bernet et al. (1987)
Pseudomonas sp. C-3	0.09-2.6	NS	NS	NS	NS	Shanker et al. (1990)
Xanthomonas maltophila	9.0	30	9.0	NS	NS	Nawaz et al. (1993)
Pseudomonas sp. NCTR 2	11.8,14.0	30	8.5	NS	NS	Nawaz et al. (1993, 1994b)
Rhodococcus sp.	NS	40	8.5	360	Ni^{2+}, Cu^{2+}, Hg^{2+}, Co^{2+}	Nawaz et al. (1994a)
Pseudomonas chloroaphis	lower than other unsaturated amides	≤ 50	7.0-8.6	105	Ag^+, Cu^{2+}, sulfhydryl reagents	Ciskanik et al. (1995)
Rhodococcus erythropolis MD50	0.94	55	7.5	480 to 500	Ag^+, Cu^{2+}, Hg^{2+}, Zn^{2+}	Hirrlinger et al. (1996)
Klebsiella pneumoniae NCTR 1	NS	65	7.0	62	Sulfhydryl reagents	Nawaz et al. (1996)
Pseudomonas aeruginosa	12.0	30	7.0	55	NS	Kumar and Kumar (1998)
Bacillus cereus	0.26	NS	7.2	NS	NS	Saroja et al. (2000)

[a] NS – not stated

[b] mol h^{-1} g^{-1} for free cells

Early studies on acrylamide degradation were concerned with the presence of acrylamide in water bodies as a result of industrial use of PAM. Croll et al. (1974) examined biodegradation of acrylamide in river water mixed microbial cultures under aerated, sunlit conditions. Following a long lag period of 220 h, they found that 8 μg L^{-1} acrylamide degraded rapidly, although no rate was stated. Acclimation of

microorganisms reduced the lag time considerably to 5 h in subsequent batch culture studies. They concluded that a microbial community capable of acrylamide degradation could be present, but cautioned that the observed lengthy lag time may pose a downstream environmental risk.

Brown et al. (1980c) also studied aqueous acrylamide biodegradation; however, they examined its fate in aerobic, illuminated river, sea, and estuarine waters. Higher concentrations of 0.5 to 50 mg L^{-1} were degraded, with lag periods ranging from 30 to 500 h. Because sterile controls showed no acrylamide loss, the removal of acrylamide was believed to be microbiological. Later studies by Brown et al. (1980b) investigated the removal of acrylamide from river water and sewage works, and found that activated sludge caused 50 to 70% removal of acrylamide in 100 h. Because treatment with mercuric chloride at 70°C inhibited acrylamide removal, the researchers concluded that the removal was microbiological. However, no microbiological testing was completed to identify the organisms responsible.

Future reclamation of TT from the oil sands necessitates knowledge of the potential acrylamide concentrations and rates of degradation in solid medium such as soil and its contained waters. Several researchers have demonstrated the ability of soil microflora to biodegrade acrylamide (Abdelmagid and Tabatabai 1982, Lande et al. 1979; Kay-Shoemake et al. 1998a). Abdelmagid and Tabatabai (1982) used an increase in ammonium concentration as proof that field soil microorganisms hydrolyzed acrylamide in soil microcosms, but did not include a sterile control to exclude abiotic hydrolysis. Lande et al. (1979) investigated the mineralization of ^{14}C-labeled acrylamide in garden soil, and found that soil communities mineralized acrylamide completely to $^{14}CO_2$, with half-lives ranging from 10 to 45 h, depending on soil type and time of sample collection. Acrylamide concentration had an effect on time required for degradation. At high concentrations (500 mg acrylamide L^{-1}), a 5-fold longer period (94.5 h) was required for complete degradation than a lower concentration (25 mg L^{-1}). In studies investigating the biodegradation of PAM, Kay-Shoemake et al. (1998a) demonstrated that a mixed culture derived from soil was able to utilize acrylamide as its sole source of carbon and nitrogen. Other researchers have investigated the biodegradability of residual acrylamide in PAM, and found that a monomer

concentration of 50 $\mu g\ L^{-1}$ was biodegraded completely in 7 d, with an acclimation period of 3 d (Soponkanaporn and Gehr 1989). Mixed cultures in both soil and water have demonstrated acrylamide degradation, although only two studies (Croll et al. 1974; Soponkanaporn and Gehr 1989) have examined a low acrylamide concentration more accurately reflecting the acrylamide level present in TT.

Semple et al. (2001) investigated the aerobic biodegradation of acrylamide at a concentration of 1 mg L^{-1} using MFT from Syncrude's Mildred Lake Settling Basin (MLSB) as an inoculum. They found that an unacclimated oil sands community degraded this acrylamide concentration, with the production and subsequent loss of acrylic acid and ammonium. Based on this finding, the microbial community present in TT should be capable of aerobic acrylamide degradation.

Pure cultures of microorganisms capable of degrading acrylamide have been isolated from environments such as garden soils, agricultural soils, sewage sludges, and effluent from industries using acrylamide or PAM (Arai et al. 1981; Kumar and Kumar 1998; Shanker et al. 1990; Wang and Lee 2001). Previous exposure to amide-containing chemicals, such as herbicides in agricultural soils, appears to stimulate communities of organisms capable of degrading acrylamide (Kumar and Kumar 1998; Nawaz et al. 1994b). Ubiquitous soil microorganisms such as *Pseudomonas* spp. (Asano et al. 1982; Ignatov et al. 1995; Kumar and Kumar 1998; Nawaz et al. 1993; Shanker et al. 1990; Wang and Lee 2001; Zabaznaya et al. 1998), *Xanthomonas maltophila* (Nawaz et al. 1993), *Alcaligenes* sp. (Zabaznaya et al. 1998), and *Rhodococcus* spp. (Arai et al. 1981; Hirrlinger et al. 1996; Ignatov et al. 1995; Nagasawa et al. 1990; Nawaz et al. 1994a) have demonstrated acrylamide-degrading abilities (Nawaz et al. 1993), as well as other microorganisms such as *Brevibacterium* sp. (Bernet et al. 1987; Ignatov et al. 1995, 1996) and *Klebsiella pneumoniae* (Nawaz et al. 1996). However, species within the same genus differ in their ability to use acrylamide. For example, only one of two strains of *Rhodococcus* spp. was able to use acrylamide in one experiment (Arai et al. 1981). Despite observed amidase inhibition caused by its vinyl group, acrylamide is a sole source of carbon and nitrogen for certain ubiquitous microorganisms, likely due to its high solubility and its accessible ammonium moiety. Acrylamide present in the pore or release water in TT may stimulate a microbial community capable of acrylamide

degradation, and as TT usage continues, any observed lag period for acrylamide degradation may be reduced due to acclimation of the microbial community.

1.3.6.4. Biodegradation of acrylamide under anaerobic conditions

Little research has been reported on the fate of acrylamide under anaerobic conditions. Acrylic acid is biodegraded under anaerobic conditions, and there is no apparent oxygen requirement for amidase activity to yield acrylic acid from acrylamide (Figure 1.4). Early oxygen-limited studies by Croll et al. (1974) report that acrylamide degraded more rapidly in a closed vessel inoculated with sewage effluent, because acrylamide was undetectable by 9 d compared to 16 d in an open vessel. Conversely, other researchers found that under more stringent anaerobic conditions, acrylamide biodegraded 9 times more slowly in soils under a nitrogen atmosphere than in soils under air (Lande et al. 1979). Brown et al. (1980c) also reported slower biodegradation of 5 mg acrylamide L^{-1} under anaerobic conditions, and further reported that degradation was significantly more rapid in illuminated cultures than in dark cultures. Conditions representative of the environment also revealed that anaerobic acrylamide biodegradation could occur, because acrylamide was found to be hydrolyzed to ammonium with a 76 to 93% efficiency in waterlogged soils (Abdelmagid and Tabatabai 1982). More recently, Wang and Lee (2001) investigated acrylamide degradation under denitrifying conditions by *Pseudomonas stutzeri*. High (440 mg L^{-1}) and low (149 mg L^{-1}) concentrations of acrylamide were removed completely, with production of acrylic acid and ammonium. Acrylamide was removed completely at the lower concentration in 16 h, whereas the degradation rate was slower at the higher concentration. Thus, relatively high acrylamide concentrations can be biodegraded under anaerobic conditions, although no work has been done with the low concentrations of residual monomer present in PAM formulations.

1.4. The need to investigate the biodegradation of low concentrations of acrylamide

Acrylamide is present at trace contaminant concentrations in commercial PAMs, with concentrations ranging from 140 to 460 $\mu g\ g^{-1}$ in commercial PAM formulations

examined in the TT application in the oil sands (Semple et al. 2001). In the TT process, PAM dosages are expected to range from 15 to 50 g m^{-3} of tailings (2 to 20% solids content). A potential loading of acrylamide to TT release waters could range from 2.5 to 25 $\mu g\ L^{-1}$. The European Chemicals Bureau (2002) estimated that for a PAM application of 500 g m^{-3} (PAM with acrylamide content of 1000 $\mu g\ g^{-1}$) in sewage treatment, the final acrylamide concentration would be 500 $\mu g\ L^{-1}$, and with an application of 0.5 g m^{-3} in drinking water the acrylamide concentration would be approximately 0.1 $\mu g\ L^{-1}$ (based on acrylamide content in PAM of 250 $\mu g\ g^{-1}$) Acrylamide concentrations in wastewaters of other industries can range from 10 to 60 $\mu g\ L^{-1}$ in pulp and paper manufacturing to 500 $\mu g\ L^{-1}$ in mineral processing (Brown et al. 1980a). The final acrylamide concentration in TT expected as a result of PAM use is comparable to most industrial applications (less than 25 $\mu g\ L^{-1}$), but higher than potable water standards, that are less than 0.5 $\mu g\ L^{-1}$.

In TT waters, in order to ensure removal of the acrylamide, biodegradation must occur at very low acrylamide concentrations. To date, pure culture biodegradation studies have examined only high acrylamide concentrations, ranging from 500 mg L^{-1} to 4500 mg L^{-1}. Several *Pseudomonas* spp. (Nawaz et al. 1994b; Shanker et al. 1990; Wang and Lee 2001) and *Rhodococcus* spp. (Nawaz et al. 1994a, 1998) demonstrated growth on concentrations as low as 500 mg L^{-1}; however, Kumar and Kumar (1998) report that an acrylamide concentration greater than 1400 mg L^{-1} is necessary for *P. aeruginosa* to demonstrate significantly greater biodegradation than in control cultures. Therefore, a minimum acrylamide concentration may be necessary for biodegradation to proceed. Although these studies provide useful information regarding the acrylamide biodegradation pathway, they do not give an indication of the biodegradation potential of acrylamide in the low part per billion (less than 25 $\mu g\ L^{-1}$) range, which is more typical of the expected acrylamide concentration in TT. In addition, all but one of these earlier experiments were conducted under aerobic conditions; the sole anaerobic study was done by Wang and Lee (2001) using *P. stutzeri* under nitrate-reducing conditions. Although TT mixes will initially be aerobic, the high oxygen demand of the TT will result in their going anoxic quickly and eventually anaerobic environments can be expected (M.D. MacKinnon, Syncrude Canada Ltd., personal communication, 2003). Thus, research on

the anaerobic biodegradation of acrylamide at $\mu g\ L^{-1}$ concentrations is needed to demonstrate that acrylamide can be degraded in TT under anoxic conditions.

1.5. Objectives of research

The main objective of this research was to predict the fate of residual acrylamide in the TT deposits in the oil sands tailings disposal. At deposition, TT are aerobic for a short time, but anaerobic conditions will develop as oxygen is consumed and as the deposit deepens. Many aerobic mixed and pure cultures degrade acrylamide, but little research has examined the role of anaerobic microorganisms in acrylamide biodegradation. Sulfate is a major electron acceptor in oil sands tailings, and tailings ponds demonstrate an active sulfate-reducing bacteria community (Holowenko et al. 2000, Foght et al. 1985). Thus, this research examined anaerobic biodegradation of ppb concentrations of acrylamide and acrylic acid under sulfate-reducing conditions, both in laboratory microcosms and in tailings under simulated field conditions. Once sulfate is depleted, methanogenic conditions will predominate, and so the effect of acrylamide on methanogenesis was also investigated. A secondary objective was to attempt to determine the stability of PAM in the TT deposits.

The research plan consisted of a laboratory component and a simulated field component. These are summarized below.

The major aims of the laboratory component were to:

a) Determine the biodegradability of acrylamide and acrylic acid under aerobic conditions using TT as sources of microorganisms.

b) Determine the biodegradability of acrylamide and acrylic acid under sulfate-reducing and methanogenic conditions using oil sands tailings and other environmental samples as sources of microorganisms.

c) Determine whether PAM can serve as a nitrogen source for microbial growth under sulfate-reducing and methanogenic conditions using oil sands tailings and other environmental samples as sources of microorganisms.

The major aim of the simulate field component was to study the biodegradation of acrylamide in microcosms established in TT.

2. Materials and Methods

2.1. Analytical Methods

2.1.1. Acrylamide and acrylic acid

Acrylamide and acrylic acid were analyzed using the high performance liquid chromatography (HPLC) method of Ver Vers (1999). A Varian Star #2 system equipped with a 330 UV-VIS photodiode array (PDA) detector set at 210 nm was used for these analyses. Two columns in series were used to separate acrylamide and acrylic acid, an ODS-AL C-18 column (150 × 4.6 mm; YMC, Kyoto, Japan) combined with a Fast-Acid ion exchange column (100 × 7.8 mm; BioRad, Hercules, CA). A guard column (Vydac 218TP, 4.6 mm, 5 μm C-18, Grace Vydac, Columbia, MD) was used to minimize degradation of the analytical columns. Standards ranging from 16 to 1000 μg L^{-1} were freshly prepared and analyzed with each set of samples from cultures, because these standards were the linear range of the method (Semple et al. 2001). The system was run isocratically, with the mobile phase of 0.01 M H_2SO_4 at a flow rate of 0.6 mL min^{-1}. A sample size of 100 μL was injected using a Varian Pro-star Model 400 autosampler. Mean retention times were approximately 10.9 and 17.0 min for acrylic acid and acrylamide, respectively. Data were analyzed using the Pro-Star 400 Star Chromatography Workstation software (Version 5.31). Prior to analysis, samples were prepared by centrifuging at 16 000 × *g* for 10 min to remove solids, then diluting in mobile phase if the expected concentration was outside of the linear range of the method.

2.1.2. Acetate

Acetate was analyzed using a Varian 3600 GC equipped with a DB-FFAP Megabore column (15 m, 0.53 mm I.D., 1 μm film thickness; J & W Scientific, Folsom, CA). Helium (5.5 mL min^{-1}) was used as the carrier gas, while nitrogen at a flow rate of 30 mL min^{-1} was the auxiliary gas. Flow rates for hydrogen and air were 30 and 300 mL min^{-1} respectively. The split flow was set to 5.1 mL min^{-1}. Injector and FID temperatures were 200°C and 250°C, while the oven temperature was 110°C isothermal.

The retention time for acetate was 2.7 min using this method. Peak areas were obtained from a HP 3380A integrator. Samples were prepared for analysis by centrifuging at 16 000 × *g* for 10 min to remove solids, and acidifying 90 µL of the supernatant with 10 µL of 4 N H_3PO_4. An injection volume of 1 µL was used. The calibration curve for acetate was linear in the concentration range of 32 mg L^{-1} to 300 mg L^{-1}, so dilution in water was performed if the expected acetate concentration was above the linear region of the calibration curve.

2.1.3. Benzoate

Benzoate was analyzed using a Waters HPLC equipped with a RP C-18 column (10 µm particle size, Brownlee Columns, Perkins Elmer, Norwalk, CT). The mobile phase consisted of 65% water, 35% acetonitrile, 10 mL 0.5 M K_2HPO_4 L^{-1}, and 2 mL 0.5 M H_3PO_4 L^{-1} at a flow rate of 1 mL min^{-1}. A Waters UV-VIS detector set to a wavelength of 230 nm was used. Using this method, the retention time of benzoate was 2.7 min. Data were collected on an HP 3390A integrator. Standards ranging from 3.1 mg L^{-1} to 100 mg L^{-1} were freshly prepared and analyzed each day that culture samples were analyzed. Samples were centrifuged at 16 000 × *g* for 10 min to remove solids, and diluted in mobile phase if the expected benzoate concentration was above the linear range of the method.

2.1.4. Methane

Methane was analyzed using the method of Holowenko et al. (2000). An HP 5700A GC fitted with a 2 m x 0.3 cm column packed with Tenax (60/80 mesh) was used. Flow rates were 30 mL min^{-1} for hydrogen, 260 mL min^{-1} for air, and 46 mL min^{-1} for nitrogen, the carrier gas. Injector and oven temperatures were 40°C, while the FID was set at 200°C. Under these conditions, the retention time for methane was ~0.23 min. Analyses were done using 0.1 mL of headspace gas from microcosms that had been vigorously shaken prior to sampling. Peak areas were obtained from a HP 3380A integrator. Calibration curves were prepared using 0.16%, 8%, and 15% (v/v) methane in 158-mL serum bottles. Results were reported as % by vol. methane.

2.1.5. Glucose

Glucose was analyzed using the 3,5-dinitrosalicylic acid (DNS) method (Miller 1959) modified for small sample volumes. The DNS reagent contained 10 g DNS L^{-1}, 300 g potassium sodium tartrate L^{-1}, and 16 g sodium hydroxide L^{-1} dissolved in water. Samples were clarified for analysis by centrifuging at 16 000 × *g* for 10 min. To assay, 125 µL of DNS reagent and 125 µL of sample supernatant were combined in a flat bottom 96 well plate (Corning Inc., Corning, NY), sealed with Thermowell™ aluminum sealing tape (Corning Inc., Corning, NY) to prevent evaporation, and heated in a 90°C water bath for 5 min. When cool, the absorbance at 540 nm (A_{540}) was read using a Spectramax Plus 384 plate reader (Molecular Devices, Sunnyvale, CA) blanked with appropriate medium and DNS reagent. Data were manipulated with Softmax Pro software, version 3.1.2 (Molecular Devices, Sunnyvale, CA). Standards ranging from 50 to 400 mg L^{-1} were freshly prepared and analyzed in duplicate with each set of culture samples. If the sample concentration was expected to be outside the range of the method, samples were diluted in the appropriate medium.

2.1.6. Lactate

Lactate in samples was derivitized using the method of Miwa and Yamamoto (1987). Samples were centrifuged at 16 000 × *g* for 10 min to remove solids. Forty-two microlitres of sample were added to 84 µL of 15.8 mM 2-nitrophenylhydrazine hydrochloride (2-NPH) (TCI America, Portland, OR), dissolved in a 3:1 95% ethanol: 0.4 M HCl solution, and 84 µl of 125 mM 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (1-EDC) (Sigma, St. Louis, MO), dissolved in a 1:1 95% ethanol: 3% pyridine (v/v) in 95% ethanol solution. Following a 20 min incubation in a 60°C water bath, 42 µL of 140 mM KOH (prepared in 4:1 methanol: distilled water) were added, then the samples were incubated for an additional 15 min at 60°C. The samples were cooled under cold water, and maintained on ice until analysis. Lactate was analyzed using a Waters HPLC equipped with a RP C-18 column (10 µm particle size, Brownlee Columns, Perkins Elmer, Norwalk, CT). The mobile phase consisted of 60% water, 40%

methanol adjusted to pH 4.4 with 2 M H_3PO_4. One microlitre injections were performed, and the flow rate was 1.2 mL min^{-1}. A Waters UV-VIS detector set to a wavelength of 400 nm was used. Using this method, the retention time of lactate was 1.9 min. Data were collected on an HP 3390A integrator. Lactate standards ranging from 223 mg L^{-1} to 2670 mg L^{-1} were prepared from a lithium lactate stock (Sigma-Aldrich, St. Louis, MO).

2.1.7. Ammonium

Ammonium was analyzed by the phenate-hypochlorite method using an automated system and a colorimetric method developed by Friedrich and Mitrenga (1981) modified for 250 μL sample volumes. For the automated method, samples were centrifuged at 16 000 × *g* for 10 min, then filtered through a 0.45 μm filter prior to analysis. Samples were analyzed on a Technicon Autoanalyzer II (Technicon Industrial Systems, Tarrytown, NY).

To assay for ammonium by the modified method of Friendrich and Mitrenga (1981), 23 μL of sample were added to 114 μL of phenol reagent containing 0.106 M phenol and 0.17 mM sodium nitroferricyanide in a flat bottom 96 well plate. After 114 μL of a reagent containing 11 mM hypochlorite in 0.125 NaOH were added, the plate was sealed with Thermowell™ aluminum sealing tape to prevent evaporation, and heated in a 30°C water bath for 30 min. When cool, the absorbance at 550 nm (A_{550}) was read using a Spectramax Plus 384 plate reader blanked with 20 mM sodium pyrophosphate buffer, pH 8.0, treated with both reagents. Data were manipulated with Softmax Pro software, version 3.1.2. Ammonium standards ranging from 49 μM to 3100 μM were freshly prepared from ammonium nitrate and analyzed in duplicate. If the sample concentration was expected to be outside the linear range of the method, samples were diluted in 20 mM sodium pyrophosphate buffer, pH 8.0.

2.1.8. Sulfate

Sulfate was analyzed by ion chromatography using a Dionex DX600 chromatograph (Dionex Ltd., Oakville, ON) equipped with an ASRS-Ultra 4 mm suppressor on recycle mode and Ionpac AS-HC 4 mm and AG-HC 4 mm columns. The eluent used was 9 mM carbonate at a flow rate of 1 mL min^{-1}. The sample loop size used

was 25 μL. Samples were centrifuged at 16 000 × *g* for 10 min and diluted in water to within the linear range of the instrument, which was 10 mg L^{-1} to 40 mg L^{-1}, and filtered through a 0.45 μm filter prior to analysis. Sulfate results were verified for several samples using the turbidimetric method of Kolmert et al. (2000).

2.1.9. Protein

Protein analysis was performed using a BCA Protein Assay Kit (No. 23225, Pierce, Rockford, IL). Samples were centrifuged at 16 000 × *g* for 10 min to remove solids. Standards were prepared from 0 to 1000 μg mL^{-1} from bovine serum albumin, fraction V (BSA) (No. 23209, Pierce, Rockford, IL). Ten microlitres of each standard and sample were dispensed into a flat bottom 96 well plate, and 200 μL of the working reagent (1 part BCA reagent B in 50 parts BCA reagent A (both Pierce, Rockford, IL) was added. The plate was sealed with Thermowell™ aluminum sealing tape to prevent evaporation, and heated in a 60°C water bath for 30 min. When cool, the absorbance at 570 nm (A_{570}) was read using a Spectramax Plus 384 plate reader blanked with culture medium treated with the reagent. Data were manipulated with Softmax Pro software, version 3.1.2.

2.1.10. Carbon analysis

Total carbon (TC) and total inorganic carbon (TIC) analyses were performed using a Dohrmann DC80 instrument equipped with a nondispersive infrared (NDIR) detector. Two hundred microlitre injections were performed, and the samples were diluted to within the linear range of the method, which was 10 to 800 mg carbon L^{-1}. For TC, potassium hydrogen phthalate was used to calibrate the equipment, while for TIC, sodium carbonate was used. Organic carbon (OC) was determined by subtracting TIC from TC.

2.1.11. Viscosity measurement

Viscosity measurements on selected PAM-containing methanogenic cultures were performed using a Cannon-Ubbelohde viscometer (Cannon Instrument Co., State College, PA). Samples (3.8 mL) were centrifuged at 16 000 × *g* for 10 min to remove particulate matter, and diluted in Milli-Q water to fall within the range of the instrument.

Results are reported as centistokes per second (cSt s^{-1}) instead of centipoise because the densities of the solutions (required to convert cSt to cp) were not known. The method was verified with glycerol standards prior to use with PAM. A standard curve was generated using PAM solutions ranging from 0% to 0.0165% PAM in water. Triplicate analyses of each sample were performed.

2.1.12. Extracellular amidase activity

Extracellular amidase activity was determined using the method described by Kay-Shoemake et al. (1998b). In an initial experiment, 1.4 mL were removed from each aerobic culture, centrifuged at 16 000 × *g* for 10 min, and placed on ice. Reaction mixtures containing 700 μL of culture supernatant and either 100 mM acetamide or 250 mg PAM L^{-1} in 20 mM sodium pyrophosphate were prepared aseptically in triplicate. Following 2 h of incubation in a 30°C water bath, samples were taken for ammonium analyses as described in Section 2.1.7.

In a later experiment, a 100 mL volume was removed aseptically from cultures inoculated with Syncrude process water, centrifuged at 16 000 × *g* for 10 min at 4°C, and placed on ice. Supernatants of the cultures containing NH_4NO_3 and no fixed nitrogen were concentrated by factors of 4 and 25 respectively using a 10 000 MW cutoff Diaflo ultrafiltration membrane in a Model 720 Ultrafiltration cell (Amicon, Oakville, ON), then sterilized by filtration through a Millex®-GV 0.22 μm low protein-binding filter (Millipore, Billerica, MA). Reaction mixtures were aseptically prepared containing 100 mM of acetamide, 100 mM acrylamide, or 250 mg PAM L^{-1} combined with 700 μL of filtered supernatant to give a 1 mL total volume. The supernatant of the culture containing PAM was too viscous to filter, and so was not filtered. Reaction mixtures containing 25 units of a commercial amidase (EC 3.5.1.4; Sigma-Aldrich, St. Louis, MO) and the test amides were also prepared as positive controls. Controls containing only buffer plus amide and buffer only were also prepared. All reaction mixtures were prepared aseptically, incubated at 30°C in a water bath, and samples aseptically removed periodically for ammonium analysis (described in Section 2.1.7) and protein analysis (described in Section 2.1.9).

2.1.13. Determination of nitrogenase activity in aerobic cultures containing PAM as nitrogen source

Nitrogenase activity assays were performed on selected cultures that had been provided with PAM as a nitrogen source (cultures described in Section 2.2.3.1) (Turner and Gibson 1980). One millilitre of culture was inoculated into 10 mL of nitrogen-deficient combined carbon medium (Rennie 1981), and cultures were incubated at 30°C. Cultures provided with glucose as carbon source in the PAM-containing enrichment cultures were provided with glucose as a carbon source in the nitrogenase assay (1000 mg L^{-1}), while enrichment cultures acclimated to acetate were provided with acetate (1000 mg L^{-1}). *Azotobacter vinelandii* (courtesy of H. Whelan, University of Alberta) was inoculated and used as a positive control for nitrogenase activity.

Headspace gas samples (0.2 mL) were taken at 24 h, 48 h, and 1 week, and reduction of acetylene to ethylene was measured using a Hewlett-Packard 5890 series II GC equipped with a thermal conductivity detector fitted with a 2 m x 0.3 cm column packed with Poropack R (60/80 mesh). Injector, oven, and detector temperatures were 37°C, 35°C, and 80°C respectively. Helium was the carrier gas at a flow rate of 59 mL min^{-1}. Under these conditions, the retention time for acetylene was ~2.9 min, while ethylene was retained on the column for ~ 5.2 min. Calibration curves were prepared using 0.16%, 8%, and 15% (v/v) acetylene plus ethylene prepared in 158-mL serum bottles.

2.1.14. Optical density (OD_{600})

OD readings were taken using a Philips PU 8740 UV-VIS spectrophotometer set to 600 nm using appropriate medium to blank the instrument.

2.1.15. Anaerobic techniques

All medium and stock solutions were prepared using standard anaerobic techniques (Balch and Wolfe 1976, Miller and Wolin 1974). Media were cooled while sparging with O_2-free gas, which was 30% CO_2, 70% N_2 for methanogenic medium and 10% CO_2, 90% N_2 for Widdel-Pfennig medium for SRB. Gases were passed over a heated copper coil to remove O_2, and were sterilized by filtering through a 0.22 μm filter

if required. Media were dispensed into 125-mL serum bottles (No. 223748; Wheaton Scientific, Millville, NJ) using O_2-free gas-flushed pipettes, and were sealed with blue butyl rubber stoppers (trimmed to ~1 cm for ease of sampling) and 13 mm aluminum seals. These serum bottles have a total volume of 158 mL (Roberts 2002). Experiments described in Section 2.2.2.3.3 used 100-mL serum bottles (No. 223747, Wheaton Scientific, Millville, NJ).

Cultures were inoculated in an anaerobic chamber (Coy Laboratory Products, Grass Lake, MI) containing an atmosphere of 5% CO_2, 10% H_2, and 85% N_2. Following inoculation, the headspaces of cultures under sulfate-reducing conditions were flushed for 2 min with sterile O_2-free gas (30% CO_2, 70% N_2) to ensure no H_2 remained to serve as a possible electron donor. Sulfide was added as a reducing agent to all anaerobic cultures to give a final concentration of 1 mM. Sampling was done with sterile 20 gauge needles fitted onto Luer-Lok disposable syringes (Becton Dickinson, Mississauga, ON) that had been flushed with sterile O_2-free gas, or was done in an anaerobic chamber to avoid introducing oxygen into the cultures. Stock solutions were boiled, sparged with O_2-free N_2 gas, and sterilized prior to addition to cultures.

2.1.16. Verification of the presence of SRB in experiments with acrylamide and acrylic acid as carbon sources

The presence of SRB in MFT, TT, and mud-containing cultures was detected by adding 1 mL of sample to 9 mL of the appropriate medium in 15-mL Hungate tubes (Belco, Vineland, NJ). Tubes were scored after 30 d incubation at room temperature in the dark. Positive tubes for SRB contained a black precipitate on the iron finishing nails.

SRB were cultivated using the method of Fedorak et al. (1987). Modified Butlin's medium (Butlin et al. 1949) containing 10 mM sodium lactate and two acid-washed iron finishing nails per tube was used.

2.1.17. Chemicals

Acrylamide (99%), acrylic acid (99%), and methyl acrylate (99%) were obtained from Sigma-Aldrich. The PAM preparation used in all biodegradation experiments was Magnafloc® LT27AG (Ciba® Specialty Chemicals, Mississauga, ON), an ultra-high

weight anionic polymer with a 29% charge density. This formulation was solvent washed by Ciba® to produce a very low (less than 10 mg kg^{-1}) residual acrylamide content (R. Schaeffer, Ciba® Specialty Chemicals, personal communication, 2002; Semple et al. 2001). Specifications of other formulations used in this study are given in Table 2.1. Chemicals used as carbon sources were glucose (BDH, Mississauga, ON), sodium acetate, sodium benzoate, and sodium lactate (all Fisher, Nepean, ON). All reagents used in HPLC mobile phases were HPLC-grade, and all water used was purified using the Milli-Q® system (Millipore, Billerica, MA).

Table 2.1. PAM formulations used in experiments.

Trade name	Magnafloc 336	Alcoflood 1235	Magnafloc LT27AG
Manufacturer	CIBA	CIBA	CIBA
Charge density (mol %)	n/a[a]	25	29
Molecular-weight classification	ultra-high	ultra-high	ultra-high
Nitrogen content (%)[b]	12.3	10.5	11.7
Carbon content (%)[b]	40.7	39.0	38.9
Acrylic acid content (mg kg^{-1})[c]	2000	1800	910
Acrylamide content (mg kg^{-1})[c]	250	150	10
Charge	anionic	anionic	anionic

[a] information not available
[b] analysis performed by Analytical Service Department, Department of Chemistry, University of Alberta
[c] Semple et al. (2001)

2.1.18. Statistical analysis

Statistical analyses were performed using the Student's t-test to determine whether statistically significant differences exist between control and viable culture methane or substrate concentrations ($p<0.05$).

2.1.19. Calculations

The general form of Buswell's equation, shown by Equation 2.1, was used to estimate the expected amounts of methane produced from given substrates (Symons and Buswell 1933). The balanced equations for calculating the methane yield of substrates used in this study are given in Table 2.2.

$C_nH_aO_bN_cS_d + [n - a/4 - b/2 + 7c/4 + d/2]\ H_2O \rightarrow [n/2 - a/8 + b/4 - 5c/8 + d/4]\ CO_2 + [n/2 + a/8 - b/4 - 3c/8 - d/4]\ CH_4 + cNH_4CO_3 + dH_2S$

Equation 2.1. Buswell's equation for theoretical methane production from substrates containing carbon, hydrogen, oxygen, sulfur, and nitrogen.

Table 2.2. Estimation of methane yield using Buswell's equation for carbon sources used.

Substrate	Buswell's equation
Acetate	$CH_3COOH \rightarrow CO_2 + CH_4$
Benzoate	$C_7H_5O_2 + 4.75\ H_2O \rightarrow 3.375CO_2 + 3.625CH_4$
Glucose	$C_6H_{12}O_6 \rightarrow 3CO_2 + 3CH_4$
Acrylamide	$C_3H_5NO + 3H_2O \rightarrow 1.25CO_2 + 1.5CH_4 + NH_4HCO_3$

Using the theoretical amount of methane from a given amount of substrate (Table 2.2), the expected volume of methane (in mL) can be calculated using the assumption that 1 mol methane = 22.4 L (at STP). From this, the anticipated volume of methane (% vol.) in the headspace of rigid containers (Nelson 1971) can be calculated using Equation 2.2.

CH_4 (% vol.) =[(a)/(a + b)] x 100

Where: a = predicted vol. (mL) methane

b = vol. (mL) headspace in microcosm

Equation 2.2. Conversion of methane volume (mL) to methane volume (% vol.) in headspace of methanogenic microcosms.

To calculate the first-order kinetic data for acrylamide removal, the method of Metcalf and Eddy (1979) was used. Data were plotted as the natural logarithm (acrylamide concentration) vs. time (d) for the zone in which degradation was linear. The slope of the line is the rate constant, k_1, given in units of d^{-1}. From this, the half-life can be calculated by: half-life (d) = $\ln(2)/k_1$.

2.2. Biodegradation studies

2.2.1. Inocula used in biodegradation studies

Inocula used were an MFT sample (composite from several depths of the MLSB), or a TT slurry sample (general composition given in Section 1.1) collected on 27 Sept. 2001 from the Syncrude Aurora site. The oil sands inocula were stored at 4°C in sealed containers prior to use. Anaerobic domestic sewage sludge samples were obtained from the Gold Bar Wastewater Treatment Plant (7.9 m. depth of digestion tank). Where indicated, biodegradation studies were carried out with these inocula.

2.2.2. Removal of acrylamide and acrylic acid

2.2.2.1. Degradation of acrylamide and acrylic acid under simulated environmental conditions

2.2.2.1.1 Effect of temperature on acrylamide degradation

In this experiment, acrylamide degradation in Aurora TT (described in Section 1.1 and Section 2.2.1) under temperature conditions simulating those of the TT deposit was assessed. Concentrations of acrylamide in the μg L^{-1} range were used, because these represent field conditions (less than 15 μg L^{-1}). TT stored at room temperature were homogenized using a drill and impellor attachment (obtained from the Department of Chemical and Materials Engineering, University of Alberta) until a uniformly viscous suspension was obtained. Three-litre portions of TT were transferred into 4-L polypropylene bottles (Nalgene), and appropriate volumes of a sterile 1000 mg L^{-1} acrylamide solution were added to give final concentrations of 100, 1000, and 10 000 μg L^{-1}. Homogenizing continued for 2 min to ensure adequate mixing of acrylamide in TT.

TT with MilliQ® water added served as a control to determine the background acrylamide concentration present in TT. Following mixing, 75 g samples were dispensed into 120 mL glass jars (Qorpak), which were capped and stored in the dark at room temperature, 4°C, or –20°C. Jars stored at –20°C were set up in duplicate to provide spares in case jars broke during freezing. A total of seven jars for each treatment were set up so that one jar could be destructively sampled at each time (0.5, 1, 2, 3, 4, 5, 6 mo). One jar was sampled for each acrylamide concentration at time zero. At each sampling time, both pore water and release water were sampled. Release water was considered to be the water that collected on the TT during storage, whereas pore water was the water that was obtained by centrifuging the TT after release water removal. All samples were frozen at –20°C prior to analyses.

2.2.2.1.2 Effect of incubation under aerobic and oxygen-limited conditions on acrylamide degradation

To measure the biodegradation rate of acrylamide and acrylic acid under aerobic and oxygen-limited conditions, 3 L of TT were homogenized in a 4-L wide-mouth polypropylene bottle, and acrylamide and acrylic acid were added to give a final concentration of 100 $\mu g\ L^{-1}$ each. This experiment was designed to reflect the oxygenic conditions present in the TT deposit immediately following deposition. The bottle was stored at room temperature in the dark. Samples were taken periodically from the surface of the tailings to give an aerobic sample, and from the bottom of the bottle to reflect anoxic conditions. The upper sample represented the aerobic environment in the TT deposit, while the bottom sample was intended to reflect the anoxic environments in the TT subsurface. A sample of TT with no added acrylamide or acrylic acid was taken to determine the background concentrations of these two chemicals. Water was removed from the solids by centrifuging, and samples were stored at –20°C until analysis.

A later experiment provided more stringent anaerobic conditions. TT were homogenized as described in Section 2.2.2.1.1, and 750 mL were transferred into each of two 1-L polypropylene bottles (Nalgene). Four glass marbles were added to each bottle to aid in mixing. One of the bottles received resazurin, a redox indicator, to verify

anaerobic conditions (final concentration of 1 mg L^{-1}), and was moved into an anaerobic chamber (described in Section 2.1.15) for the duration of the experiment. The bottles were allowed to equilibrate for 1week with the lids loosened, and the bottles were shaken vigorously daily to maintain fluidity. On d 7, acrylamide was added to give a final concentration of 100 μg L^{-1}, and the bottles were shaken to evenly distribute the acrylamide. Ten millilitre samples were taken, placed in a 15-mL centrifuge tube (Corning Inc.), and centrifuged at 10 000 × *g* for 10 min. One millilitre of the supernatant was removed and stored at –20°C until analysis for acrylamide. Both bottles were incubated at room temperature, and samples were taken biweekly.

To ensure that acrylamide is not adsorbed by the polypropylene bottles, 100 μg L^{-1} acrylamide was added to 10 mM phosphate buffer in 1-L polypropylene bottles (Nalgene), and samples were taken for acrylamide analysis.

2.2.2.2. Acrylamide biodegradation under aerobic conditions in shake-flask cultures

Enrichment cultures for aerobic microorganisms capable of using acrylamide as either a sole carbon or sole carbon and nitrogen source were established. Sterile shake flasks (500 mL) were prepared containing 200 mL of sterile B + NP medium (Fedorak and Westlake 1984) for cultures in which acrylamide was the sole carbon source, with NH_4Cl replaced with KNO_3 to an equivalent nitrogen concentration to allow for ammonium detection. For cultures in which acrylamide provided both carbon and nitrogen, 0.8% NaCl was used as the medium. Filter-sterilized acrylamide was added to achieve a final concentration of 1 mg L^{-1}, and was added following medium sterilization to avoid thermal degradation. The inoculum used was the TT slurry described in Section 2.2.1. Ten millilitres of homogenized TT were aseptically added to the medium, and the flasks were shaken at 200 rpm on a rotary shaker at 28°C in the dark. Sterile controls were prepared by inoculating medium, autoclaving for 1 h d^{-1} on 3 consecutive d, and adding acrylamide. Once the acrylamide had been depleted from a culture, 10 mL of the culture were transferred to fresh medium supplemented with acrylamide. Liquid samples were taken for acrylamide (Section 2.1.1) and ammonium (Section 2.1.7)

analyses, and OD_{600} analysis (Section 2.1.14) was performed on selected samples. All samples were stored at –20°C until analyses were done.

2.2.2.3. Acrylamide biodegradation under sulfate-reducing conditions

2.2.2.3.1 Acrylamide biodegradation in high sulfate medium

Because sulfate is prevalent in oil sands tailings, sulfate-reducing conditions are expected to predominate once oxygen has been depleted. To assess the potential for biodegradation of acrylamide and acrylic acid under sulfate-reducing conditions, anaerobic microcosms were established in triplicate using the anaerobic techniques described in Section 2.1.15. Each microcosm contained 75 mL of Widdel-Pfennig medium (Collins and Widdel 1986) and acrylamide or acrylic acid, or both, each at 7 mg L^{-1}. Sterile controls were prepared by inoculating medium (inocula described below), autoclaving 3 consecutive d for 1 h d^{-1}, and adding substrates to achieve a final concentration of 7 mg L^{-1} each. Sulfate was added to achieve a final concentration of 2.3 g L^{-1}. To assess abiotic degradation of the substrates, uninoculated controls were prepared containing medium with only the test substrates.

Inocula used a mud sample (collected from Hawrelak Park, Edmonton, AB on 9 June 2002), MFT, and TT (both described in Section 2.2.1). Twenty-five grams of the mud or MFT, or 25 mL of TT were added to the serum bottles. After inoculation, microcosms were incubated at room temperature in the dark. Samples were removed from the microcosms weekly and stored at –20°C until analyses for acrylamide (Section 2.1.1), acrylic acid (Section 2.1.1), and sulfate (Section 2.1.8) were done. Cultures were transferred into medium containing fresh substrate when the substrates were depleted.

2.2.2.3.2 Acrylamide biodegradation in low sulfate medium

To enable detection of sulfate loss in the cultures, the concentrations of acrylamide and acrylic acid were increased to 71 mg L^{-1} for the third transfer of the experiment described in Section 2.2.2.3.1. Based on the balanced equations for acrylamide and acrylic acid degradation shown in Equation 2.3 and Equation 2.4, the concentration of sulfate in the Widdel-Pfennig medium (Collins and Widdel 1986) was

decreased to 3 mM (290 mg L^{-1}) sulfate for cultures containing a single substrate and to 6 mM (580 mg L^{-1}) for cultures containing both substrates.

$$2C_3H_5ON + 3SO_4^{2-} \rightarrow 6CO_2 + 2NH_3 + 2H_2O + 3S^{2-}$$

Equation 2.3. Acrylamide mineralization under sulfate-reducing conditions.

$$2C_3H_4O_2 + 3SO_4^{2-} \rightarrow 6CO_2 + 4H_2O + 3S^{2-}$$

Equation 2.4. Acrylic acid mineralization under sulfate-reducing conditions.

Serum bottles containing the cultures were incubated in an anaerobic chamber at room temperature in the dark.

2.2.2.3.3 Acrylamide biodegradation with molybdate added as an inhibitor of SRB

To demonstrate that acrylamide degradation was coupled to sulfate reduction, cultures free of sulfate and cultures containing molybdate to inhibit SRB were tested. Fifteen millilitres of MFT were inoculated into 90 mL of Widdel-Pfennig medium (Collins and Widdel 1986). Filter-sterilized acrylamide, sulfate, and molybdate were added to designated cultures to achieve concentrations of 7 mg L^{-1}, 290 mg L^{-1}, and 21 g L^{-1} respectively. Sterile controls were prepared by inoculating medium, autoclaving 3 consecutive d for 1 h d^{-1}, then adding acrylamide and sulfate to achieve final concentrations of 7 mg L^{-1} and 290 mg L^{-1} respectively. Samples were taken weekly for acrylamide and sulfate analyses were stored at –20°C until analysed. Cultures were transferred into medium containing fresh substrate when the substrates were depleted.

2.2.2.4. Acrylamide biodegradation under methanogenic conditions

2.2.2.4.1 Enrichment cultures

Once sulfate is depleted in the oil sands tailings, methanogenic conditions will predominate (Fedorak et al. 2002). To assess the potential for biodegradation of acrylamide under such conditions, methanogenic microcosms were established in triplicate using the anaerobic techniques described in Section 2.1.15. Acrylamide was

used as a carbon source at a final concentration of 7 mg L^{-1} or 71 mg L^{-1}. Sterile controls were prepared by inoculating medium, autoclaving 3 consecutive d for 1 h d^{-1}, and adding filter-sterilized acrylamide to achieve a final concentration of 7 mg L^{-1} or 71 mg L^{-1}. To account for methane production from the inoculum, inoculated controls containing no acrylamide were prepared. After inoculation, serum bottles containing the cultures were incubated in an anaerobic chamber at room temperature in the dark.

Inocula used were an anaerobic domestic sewage sludge sample (collected 22 April 2003), MFT, and TT (described in Section 2.2.1). Twenty-five millilitres of each inoculum was added to appropriate serum bottles. Liquid samples were taken weekly for acrylamide analysis, and were stored at –20°C until analysis. Headspace gas samples were taken weekly, and were analyzed for methane using the method described in Section 2.1.4. When the substrate was depleted from the cultures, a portion of the culture was transferred into fresh medium containing more acrylamide.

2.2.2.4.2 · Inhibition studies with acrylamide in methanogenic consortia

To determine whether acrylamide inhibited methane production, an inhibition assay was established using anaerobic domestic sewage sludge (Section 2.2.1, collected 6 October 2003). Prior to use, the inoculum was allowed to incubate and vent in a fume hood for 10 d to reduce the volume of methane originating from indigenous sources (carbon sources in the inoculum). Anaerobic microcosms were established in triplicate in the medium of Fedorak and Hrudey (1984) using the anaerobic techniques described in Section 2.1.15. Ten millilitres of sewage sludge were inoculated into each culture, and headspaces were flushed for 2 min with sterile O_2-free gas (30% CO_2, 70% N_2) to ensure no H_2 remained to serve as a possible electron donor. Acetate (as the sodium salt) was provided to all cultures as a readily-useable energy and carbon source at a concentration of 1000 mg L^{-1}. Concentrations of acrylamide tested were 0, 7, 40, 70, 360, and 710 mg L^{-1} to determine the extent of inhibition of methane production caused by acrylamide. After inoculation, cultures were incubated at room temperature in the dark, and samples were taken weekly for acetate and acrylamide analyses. Samples were stored at –20°C prior to analysis. Methane analysis was performed weekly as described in Section 2.1.4.

2.2.2.4.3 Attempts to demonstrate enhanced methanogenesis from acrylamide biodegradation

Exposure to acrylamide in the inhbition study (Section 2.2.2.4.2) produced an acclimated consortium which was able to overcome acrylamide toxicity. To determine whether acrylamide stimulated methanogenesis, additional experiments were performed on the acclimated consortia from the inhibition study. Once acrylamide had been completely removed in these cultures, the headspaces were flushed with sterile O_2-free gas (30% CO_2, 70% N_2) until the methane concentration was nil, and acrylamide was added to the appropriate original concentration (0, 7, 40, 70, 360, and 710 mg L^{-1}). No acetate was added to these cultures, because acrylamide was meant to serve as the carbon source for methanogenesis. Following this flushing procedure, acrylamide in the liquid phase and methane in the headspace were monitored weekly, and when the acrylamide was completely removed, the cultures were supplemented again with acrylamide, but no headspace flushing was done.

2.2.3. PAM as nitrogen source for microorganisms

2.2.3.1. PAM as a nitrogen source under aerobic conditions

Enrichment cultures for aerobic microorganisms capable of using PAM as a nitrogen source were established. Sterile shake flasks (500 mL) were prepared containing 200 mL of sterile mineral salts medium (Kay-Shoemake et al. 1998a) supplemented with glucose (1000 mg L^{-1}) or acetate (1000 mg L^{-1}) as a carbon source. Filter-sterilized glucose was added following medium sterilization to avoid loss due to carmelization. A 0.3% (w/v) PAM solution was prepared by dissolving the solid PAM in sterile MilliQ® water, using sterile glassware and a sterile stirrer, and mixing the solution for at least 0.5 h. PAM was aseptically added to give a carbon:nitrogen mass ratio of 20:1 in the medium. The inocula used were a garden soil sample (University of Alberta) and a Syncrude process water sample (1 m surface zone collected in 2001 from West In Pit (WIP)-, settling basin) stored at 4°C until use. Ten grams (garden soil) or 10 mL (process water) were added to the medium, and the flasks were shaken at 200 rpm on a rotary shaker at 28°C in the dark. Weekly transfers were performed by transferring 10

mL of a 1-week-old culture to fresh medium. Following 24 weeks, the cultures were transferred biweekly. Initially, three flasks for each inoculum were set up, but only those flasks demonstrating visual growth were maintained after 24 weeks. At 24 weeks, the OD_{600} and pH were measured to follow growth, and samples were taken for acetate and glucose loss, and ammonium production. All samples were stored at -20°C until analysis.

The amidase activity of the enrichment cultures was investigated using the method described in Section 2.1.12. An initial experiment included both garden soil and Syncrude process water enrichment cultures. Two millilitres of established enrichment cultures (grown and serially transferred for 21 mo) were inoculated into 25 mL of sterile mineral salts medium (Kay-Shoemake et al. 1998a) containing 1 g glucose L^{-1} and a volume of sterile 0.3 % (w/v) PAM or NH_4NO_3 to give a C:N mass ratio of 6:1. Inoculated medium containing no added nitrogen was also tested to determine the extent of amidase activity resulting from nitrogen carryover from the enrichment cultures. Cultures were incubated at 28°C on an orbital shaker set to 200 rpm for 72 h prior to analysis for amidase activity.

A later experiment attempted to increase the amount of extracellular amidase by concentrating a larger supernatant volume. Ten millilitres of the established Syncrude process water enrichment cultures (grown and serially transferred for 23 mo) were inoculated into 200 mL of sterile mineral salts medium (Kay-Shoemake et al. 1998a) containing 1 g glucose L^{-1} and volumes of sterile 0.3 % (w/v) PAM or NH_4NO_3 to give a carbon:nitrogen (C:N) mass ratio of 6:1. Inoculated medium containing no added nitrogen was also tested to determine the extent of amidase activity resulting from nitrogen carryover from the enrichment cultures. Cultures were incubated at 28°C on an orbital shaker set to 200 rpm for 96 h prior to analysis for amidase activity.

2.2.3.2. PAM as a nitrogen source under sulfate-reducing conditions

To assess the ability of SRB to use PAM as a nitrogen source, anaerobic microcosms were established in triplicate using the anaerobic techniques described in Section 2.1.15. Each microcosm contained 75 mL of Widdel-Pfennig medium, excluding ammonium chloride and the vitamin solution, to ensure that PAM is the sole

nitrogen source. Sodium lactate was added to give a final concentration of 2700 mg L^{-1}, and a volume of a PAM solution was added to give a final C:N mass ratio of 20:1.

Inocula used were MFT and TT, described in Section 2.2.1. Twenty-five grams of MFT or TT were added to separate microcosms. Sterile controls were prepared by inoculating medium and autoclaving three consecutive days for 1 h d^{-1}. To assess abiotic degradation of the substrates, uninoculated controls were prepared containing medium with only the test substrates. Inoculated cultures with no added nitrogen were also prepared to determine substrate loss due to residual nitrogen in the inoculum. After inoculation, samples were incubated at room temperature in the dark in an anaerobic chamber. Samples were taken biweekly for lactate and sulfate analyses, and PAM-N analysis by acid hydrolysis (Section 6.1.2). Samples were stored at –20°C until analyses were completed.

2.2.3.3. PAM as a nitrogen source under methanogenic conditions

Methanogenic microcosms using the medium of Fedorak and Hrudey (1984) were established to assess the ability of methanogenic communities to use PAM as a nitrogen source. Nitrogen sources such as the vitamin B solution, ammonium chloride, and ammonium molybdate were excluded from the medium to limit the nitrogen source to only PAM. The inocula used in enrichment cultures were an anaerobic domestic sewage sludge sample, MFT, and TT (see Section 2.2.1). Benzoate, acetate, or glucose was added as a carbon source (1000 mg L^{-1}), and PAM was added to give a final C:N mass ratio of 20:1. PAM was added following autoclaving to avoid thermal degradation. Liquid samples were taken weekly after vigorously shaking the cultures, and were stored at –20°C until analysis for benzoate, acetate, and glucose. Headspace gas samples were taken weekly, and were analyzed immediately for cumulative methane production. Ten-millilitre portions of the cultures were transferred into medium containing fresh substrate once the carbon sources had been depleted.

To verify that PAM was serving as a nitrogen source, established enrichment cultures inoculated with sewage sludge with acetate as the carbon and energy source were chosen for further study. Ten-millilitre volumes were transferred into 75 mL of methanogenic medium (Fedorak and Hrudey 1984) containing acetate (1000 mg L^{-1}),

sufficient volumes of PAM or NH_4Cl solutions to give a final concentration of 20 mg N L^{-1}. Sterile controls and controls containing no added nitrogen were also prepared. Samples were taken weekly for PAM-N analysis by acid hydrolysis and for acetate analysis.

3. Results and discussion

3.1. Biodegradation studies

3.1.1. Removal of acrylamide and acrylic acid

3.1.1.1. Degradation of acrylamide and acrylic acid under simulated environmental conditions

To assess biodegradability of acrylamide and/or acrylic acid under conditions similar to those in TT deposits, these compounds were added directly to the TT slurry described in Section 2.2.1. Their concentrations were monitored from samples of release water (the aqueous phase released during tailings storage) and pore water (water centrifuged from the solid TT phase) during incubation under various conditions.

3.1.1.1.1 Effect of temperature on acrylamide degradation

Because of the potential toxicity of acrylamide (Section 1.3.3), the degree and rate of acrylamide degradation in the TT deposit is of interest to assess risks associated with tailings disposal. To assess the persistence of acrylamide in the deposited materials under environmental conditions, 75-mL portions of a slurry of TT amended with low concentrations of acrylamide were incubated at room temperature under static conditions (approximately 22°C), 4°C, and –20°C to simulate environmental temperatures in the oil sands region of northern Alberta. In this experiment, no manipulation of oxygen concentration was performed, so the oxic conditions reflect those which likely would occur naturally in the TT deposit. No background acrylamide was detected in TT prior to acrylamide addition. Thus, all acrylamide detected originated from acrylamide addition to final concentrations of 160, 1500, and 15 000 μg acrylamide L^{-1} TT. Under these simulated conditions where oxygen was likely not limiting, rapid degradation of acrylamide in the μg L^{-1} concentration range occurred at room temperature and at 4°C (Figure 3.1, Figure 3.2, Figure 3.3). In tailings supplemented with 160 μg acrylamide L^{-1}, all of the acrylamide was removed by the first sampling time at 14 d when the TT was incubated at 4°C or 22°C (Figure 3.1). This complete removal within 14 d occurred in

both the release and pore waters, as seen in Figure 3.1a and b. Little acrylamide removal was observed in samples incubated at –20°C. Similar results were obtained in TT containing 10 times as much acrylamide (1600 $\mu g\ L^{-1}$). Acrylamide was completely removed from the release and pore waters by the first sampling date at 14 d (Figure 3.2a and b). Acrylamide persisted in only TT incubated at –20°C, as incubation temperatures of 4°C and 22°C caused rapid degradation in 14 d. However, some acrylamide loss was noted at –20°C (~40%) (Figure 3.1, Figure 3.2). The samples were thawed at room temperature only until liquid enough to homogenize fully (~30 min), and so the short time at room temperature during handling likely did not allow biodegradation to occur to account for this decrease. Thus, the reason for the decrease in acrylamide concentration is unknown. At 22°C, 15 000 μg acrylamide L^{-1} (100% of the added acrylamide) was removed in 14 d in release water (Figure 3.3a), but to see similar loss in the pore waters, 30 d was required (Figure 3.3b). After 14 d, 390 μg acrylamide L^{-1} remained in the pore water fraction (~3% of the added acrylamide). Removal at 4°C was slower in both release water and pore water, with only 31% and 35% removal observed by d 14 (Figure 3.3a and b). The degradation rates at 4°C were similar between release and pore water, with zero-order rates of 330 and 380 μg acrylamide $L^{-1}\ d^{-1}$ from d 0 to 14. These rates slowed slightly following d 14, with 220 and 210 μg acrylamide $L^{-1}\ d^{-1}$ being removed in release water and pore water respectively. As was observed in the two lower concentrations, an incubation temperature of –20°C inhibited acrylamide degradation at this higher concentration.

In this preliminary experiment, the acrylamide biodegradation occurred more quickly than expected, and only a few samples were taken during the incubation period (90 d). The measured acrylamide concentrations allowed the calculation to estimate zero-order rate constants. These are summarized in Table 3.1.

These data suggest that *in situ*, the low acrylamide concentrations expected from PAM in TT will be degraded rapidly during those portions of the year when the temperature is above 4°C. However, degradation may become slower as oxygen is consumed in the deposit. Also, as the deposit deepens, degradation may slow with depth, causing acrylamide to persist in pore waters. An experiment using a larger volume of TT

was then undertaken to sample both the aerobic surface, which is reflective of the more aerobic surface of the TT deposit, as well as subsurface environments, which may be oxygen-limited.

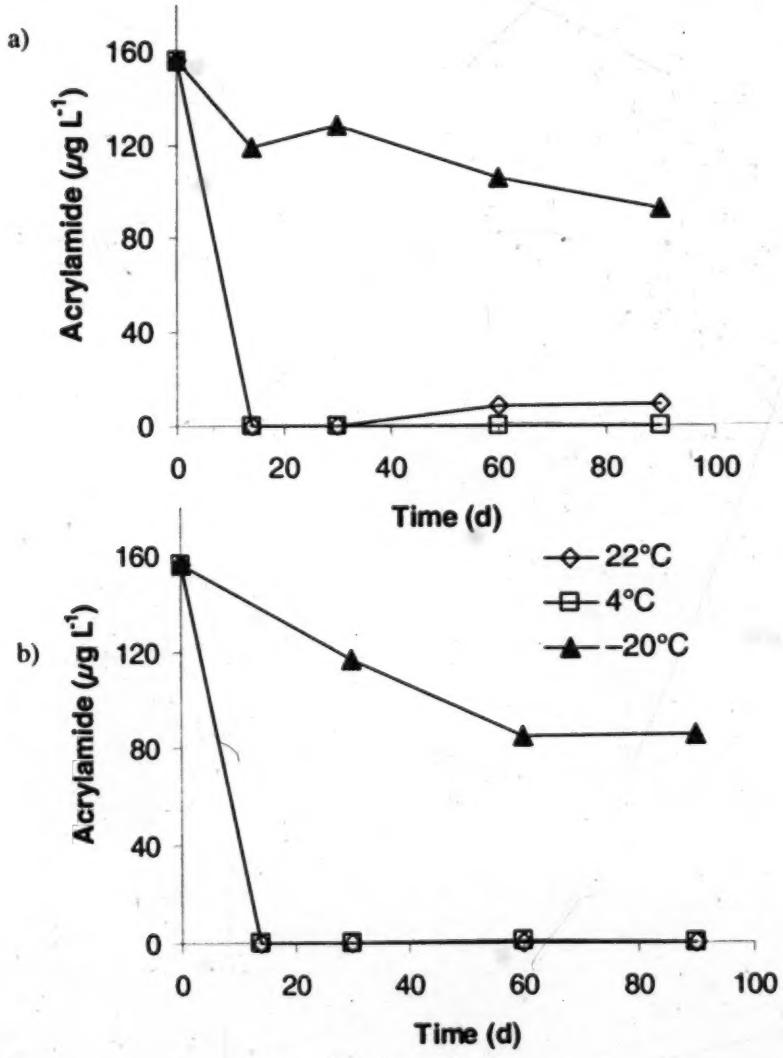

Figure 3.1. Concentrations of acrylamide in a) release water and b) pore water from TT supplemented with 160 µg acrylamide L^{-1} and incubated under static conditions.

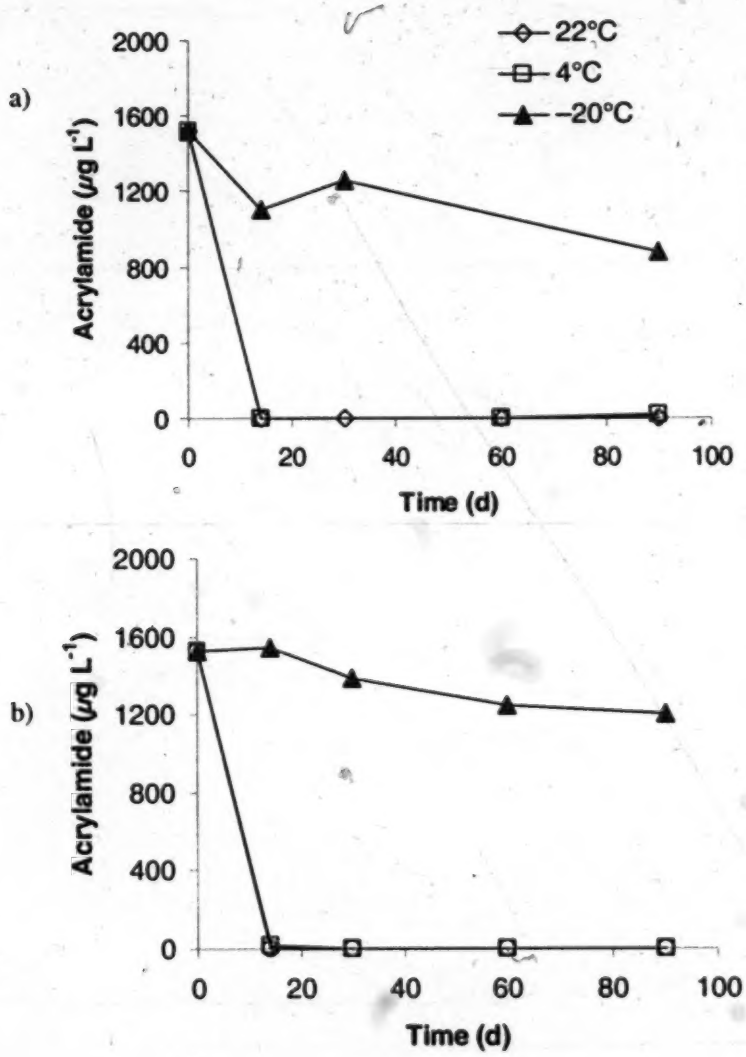

Figure 3.2. Concentrations of acrylamide in a) release water and b) pore water from TT supplemented with 1600 µg acrylamide L^{-1} and incubated under static conditions.

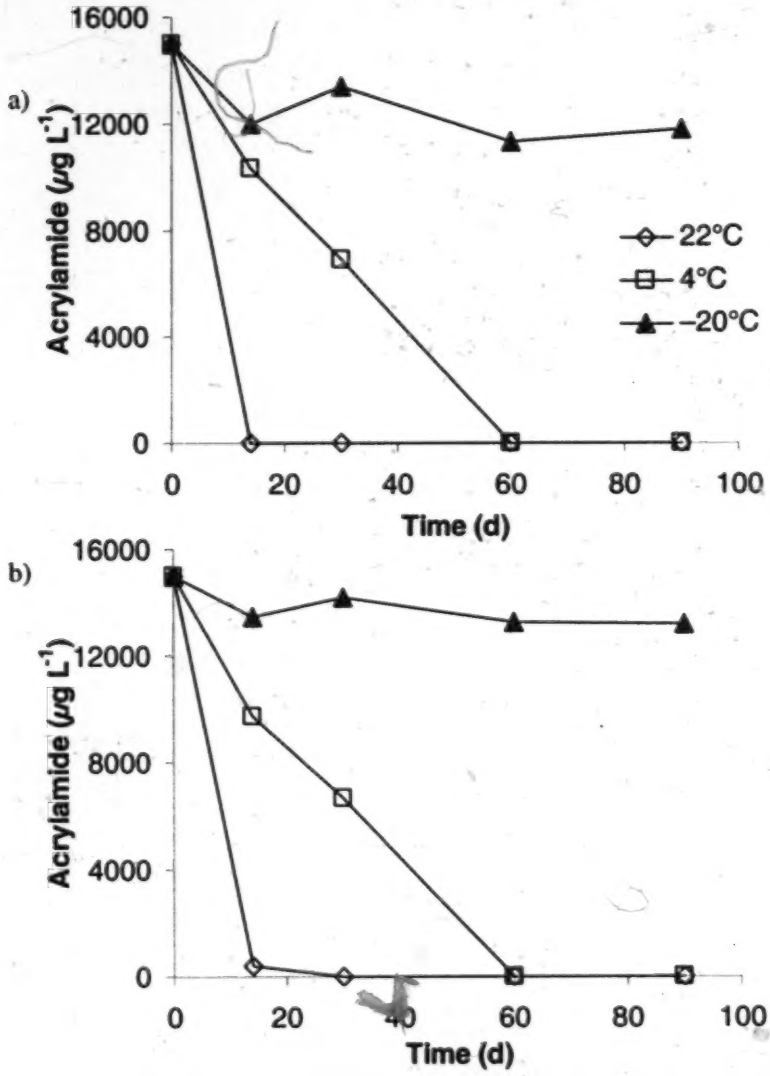

Figure 3.3. Concentrations of acrylamide in a) release water and b) pore water from TT supplemented with 15 000 µg acrylamide L^{-1} and incubated under static conditions.

Table 3.1. Summary of kinetic data for acrylamide degradation in TT under different incubation temperatures.

Initial concn. ($\mu g\ L^{-1}$)	Zero-order degradation rate ($\mu g\ L^{-1}\ d^{-1}$)			
	Release water		Pore water	
	22°C	4°C	22°C	4°C
160	> 11	> 11	> 11	> 11
1600	> 110	> 110	> 110	> 110
15 000	> 1070	170	> 1070	250

3.1.1.1.2 Effect of incubation under aerobic and oxygen-limited conditions on acrylamide degradation

The small-volume (75 mL) TT experiment did not allow for sampling at lower depths, which would be useful in determining if any differences exist between near-surface and subsurface acrylamide degradation. Two additional experiments were undertaken to elucidate any differences in acrylamide degradation rates resulting from oxygen content. In a preliminary experiment, one 4-L polypropylene jar filled with 3 L of a TT slurry incubated at room temperature was used. Near-surface and subsurface sampling were done using a wide-mouth 25 mL pipette, although increased tailings densification at later time points required using a 60 mL syringe attachment instead of a pipette bulb to provide more vacuum. Near-surface and subsurface samples were taken ~16 cm and ~6 cm respectively from the bottom of the jar, which was approximately 4 cm and 14 cm from the top of the TT .

Neither acrylamide nor acrylic acid was detected in unspiked TT at time zero. Figure 3.4 gives the results of this initial experiment. Acrylic acid was very rapidly degraded to undetectable concentrations at both the near-surface and the subsurface levels 1 d following monomer addition. Approximately half of the added acrylamide was degraded by d 5, with 59 $\mu g\ L^{-1}$ remaining at the near-surface and 63 $\mu g\ L^{-1}$ in the subsurface. Complete degradation was observed by d 14. A preliminary experiment determined that acrylamide was not adsorbed by the polypropylene bottles used in this experiment.

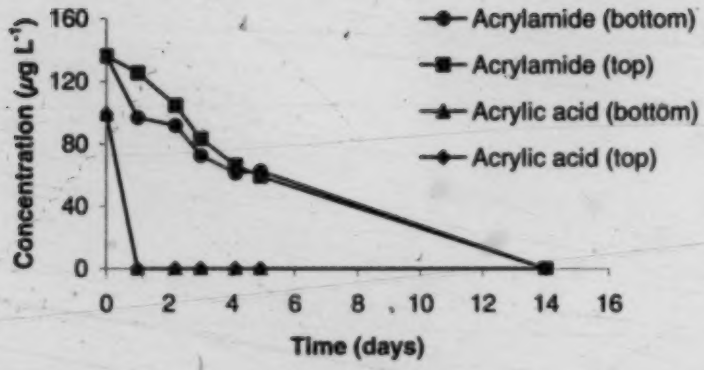

Figure 3.4. Concentration of acrylamide and acrylic acid in pore water recovered from the near-surface (top) and subsurface (bottom) of TT incubated in a 4-L jar under static conditions.

These results from the preliminary experiment reveal slight differences in degradation rates between near-surface and subsurface zones during the first 6 d, but further work was needed to conclusively elucidate any significant differences. Insufficient data were collected in this experiment to apply kinetic analysis. A second experiment incorporating more stringent oxygen-limited conditions was done by adding acrylamide to a smaller volume of a TT slurry, and maintaining this culture in an anaerobic chamber. Stringent anaerobic conditions and frequent sampling enabled for a more accurate estimate of the degradation rate of acrylamide in oil sands TT under a potentially oxygen-limited environment. Resazurin, used as an indicator of anaerobic conditions, remained colorless throughout the experiment, verifying that the TT remained anaerobic. An aerobic culture was maintained outside the anaerobic chamber (thereby not oxygen-limited) to estimate the aerobic degradation rate.

In order to estimate the degradation rate, the data were fit to show first-order kinetics. As can be seen in Figure 3.5a, both aerobic and anaerobic incubations demonstrated first-order kinetics from d 0 to 2, and following d 2, little loss was observed in either culture. At d 14, total loss was observed in aerobic cultures (Figure 3.5a). The interval of first-order kinetics (0 to 2 d) is shown in Figure 3.5b. As can be seen in Figure 3.5a and b, aerobic and anaerobic incubations of the tailings produced

similar plots. Using the method described in Section 2.1.19, the rate constant (k_1) and the half-life (in d) were determined, and are given in the first two columns of Table 3.2.

In two later experiments, the initial concentrations of acrylamide were much lower, at approximately 200 $\mu g\ L^{-1}$, which more closely approximates the concentrations expected as a result of PAM use in field applications where acrylamide concentrations in TT release and pore waters are expected to be less than 20 $\mu g\ L^{-1}$. Both trials at the low acrylamide concentrations produced similar trends, and so the plots for only trial 2 are shown (Figure 3.6). From d 0 to 7, acrylamide was removed at the same rate under aerobic and anaerobic conditions (Figure 3.6a), but after this point, acrylamide was removed rapidly under aerobic conditions (100% removed in 14 d), but persisted in the anaerobic zone (90% removed in 28 d). Again, kinetic data reveals that the rates of removal are similar under both conditions (Figure 3.6b). Acrylamide was completed removed under aerobic conditions in both trials (d 14 and d 9), while ~28 μg acrylamide L^{-1} remained under anaerobic conditions in both trials (data not shown). The kinetic data are given for both trials in Table 3.2.

The initial acrylamide concentration had an effect on the kinetics of acrylamide degradation (Table 3.2). The higher initial concentration of acrylamide had a higher rate constant and a shorter half life than did the concentrations which were ~6 times lower. Aerobic and anaerobic conditions at the same acrylamide concentration did not have a considerable effect on the half-life of acrylamide in TT, as all values were quite similar (Table 3.2).

Table 3.2. Summary of kinetic data for acrylamide degradation in TT under aerobic and anaerobic conditions.

	Acrylamide (~1200 $\mu g\ L^{-1}$)		Acrylamide (~200 $\mu g\ L^{-1}$) (Trial 1)		Acrylamide (~200 $\mu g\ L^{-1}$) (Trial 2)	
	k_1 (d^{-1})	Half-life (d)	k_1 (d^{-1})	Half-life (d)	k_1 (d^{-1})	Half-life (d)
Aerobic	1.4	0.50	0.24	2.9	0.29	2.4
Anaerobic	1.4	0.48	0.31	2.3	0.33	2.1

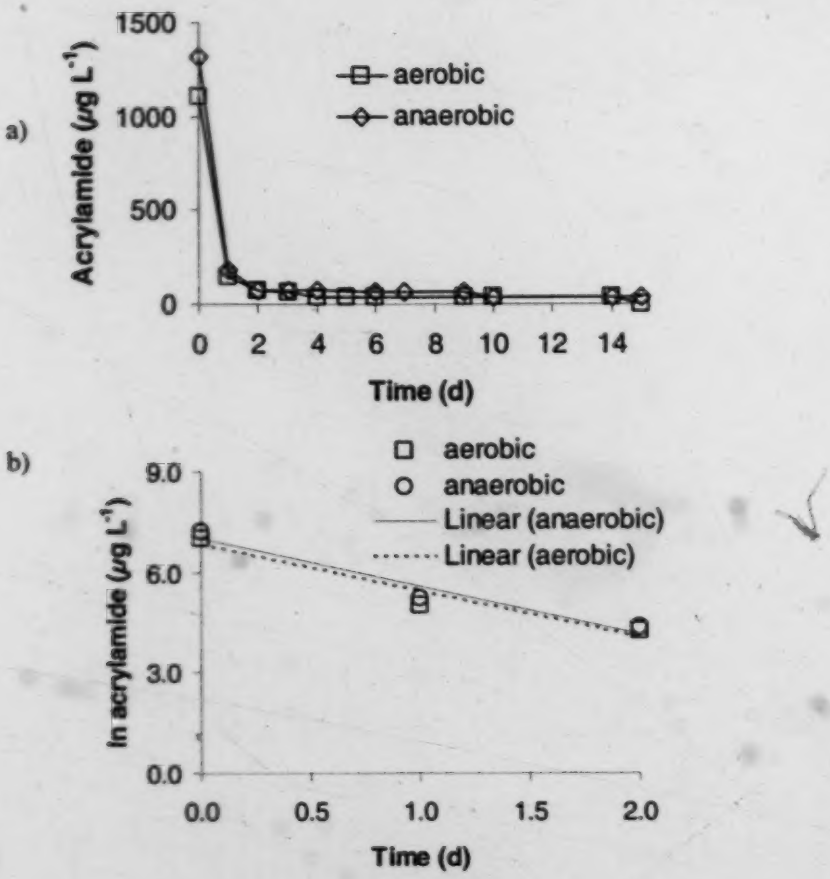

Figure 3.5. Concentration of acrylamide in pore water from TT incubated at room temperature under simulated surface (aerobic) and subsurface (anaerobic) field conditions. a) over the course of the experiment, b) showing the interval of first-order kinetics (0 to 2 d). Equations for the trendlines are $y=-1.39x+6.80$, $r^2=0.94$ (aerobic); $y=-1.43x+7.01$, $r^2=0.96$ (anaerobic).

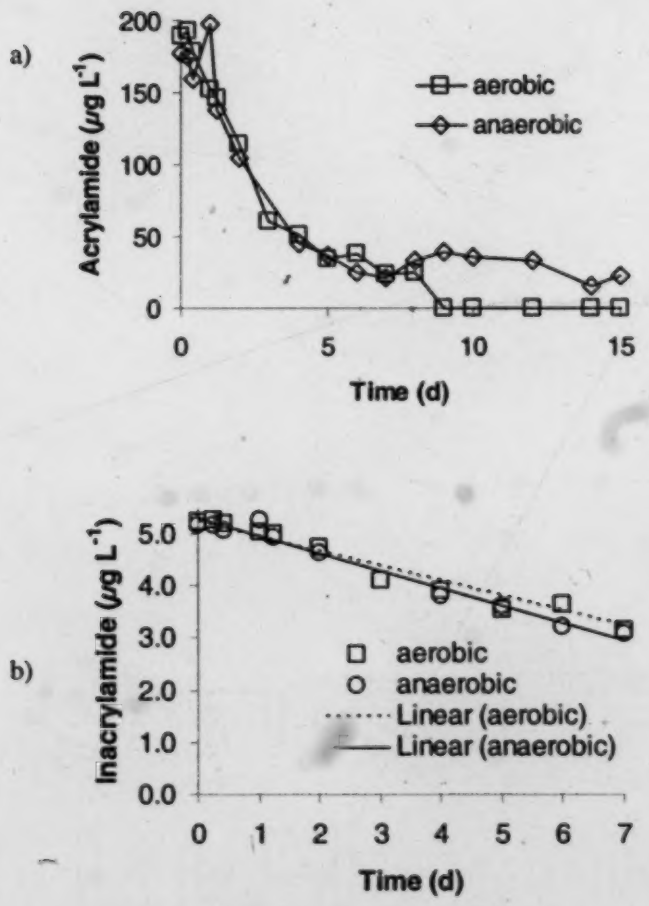

Figure 3.6. Concentration of acrylamide in pore water from TT incubated at room temperature under simulated surface (aerobic) and subsurface (anaerobic) field conditions in trial 2. a) over the course of the experiment, b) showing the zone of first-order kinetics (0 to 7 d). Equations for the trendlines are $y=0.29x+5.2$, $r^2=0.96$ (aerobic); $y=0.33x+5.3$, $r^2=0.98$ (anaerobic).

3.1.1.2. Acrylamide biodegradation under aerobic conditions in shake-flask cultures

Immediately following deposition, aerobic conditions will predominate because of the mechanical agitation involved in the Clark Hot Water Process of oil extraction, tailings transport, and the additional agitation during PAM addition to the TT mixture in the thickener. Semple et al. (2001) reported that acrylamide was readily degraded under aerobic conditions in 4 d by several sources of environmental microorganisms, including Syncrude MFT. Although results were promising, several factors confound the application of these results to polymer-treated TT deposits. At the time of the experiments performed by Semple et al. (2001), TT originating from Aurora were not available as a source of microorganisms, so MFT were used. Thus, the activity of the microorganisms from two different sources may differ, because the chemical treatment of MFT (no PAM) and TT (PAM-treated) differed. In addition, the acrylamide concentrations used (50 to 200 mg acrylamide L^{-1}) were considerably higher than those expected in field conditions (less than 20 $\mu g\ L^{-1}$). Acrylamide was present as an unreacted monomer in selected PAM formulations in concentrations ranging from 190 to 440 μg acrylamide g^{-1} polymer (Semple et al. 2001), which would correspond to low concentrations in TT deposits. Thus, their results at higher acrylamide concentrations may not be indicative of the degradation rate of acrylamide under aerobic conditions in TT deposits. To more accurately calculate acrylamide degradation rates, indigenous microbial consortia from TT and more realistic acrylamide concentrations were used.

To assess the biodegradability of acrylamide by the aerobic microbial consortia in TT, a TT slurry was used to inoculate medium containing added nutrients (B + NP medium) or medium lacking added nutrients (0.8% saline). Acrylamide was provided as the sole source of carbon in both media, and also served as a nitrogen source in the saline medium. Cultures were sampled to follow acrylamide degradation over time.

Acrylamide was readily degraded by microorganisms originating from TT deposits, as shown in Figure 3.7. When inoculated into medium supplemented with nitrogen and phosphorus, TT-inoculated cultures removed acrylamide, as observed in Figure 3.7a. By 1 d post-inoculation, 70% of the added acrylamide (650 $\mu g\ L^{-1}$) had

been removed, and the acrylamide concentration was below the detection limit by 3 d. The lag time was less than 0.5 d and the removal rate increased from 9 μg acrylamide L^{-1} h^{-1} in the first 0.5 d to 22 μg acrylamide L^{-1} h^{-1} from in the period from 0.5 d to 1.5 d. In 0.8% saline medium that contained no added inorganic nutrients, acrylamide removal progressed more slowly, as demonstrated in Figure 3.7b. After a lag phase of 3 d, acrylamide removal began with an initial rate of 6.3 μg acrylamide L^{-1} h^{-1}, which continued to 8 d. However, following 8 d, the rate slowed to less than 1 μg acrylamide L^{-1} h^{-1}. By 6 d post-inoculation, only 46% of the added acrylamide had been removed, and by 13 d, 87% removal had occurred. At 28 d, 0.05% of the initial acrylamide concentration (50 μg L^{-1}) remained.

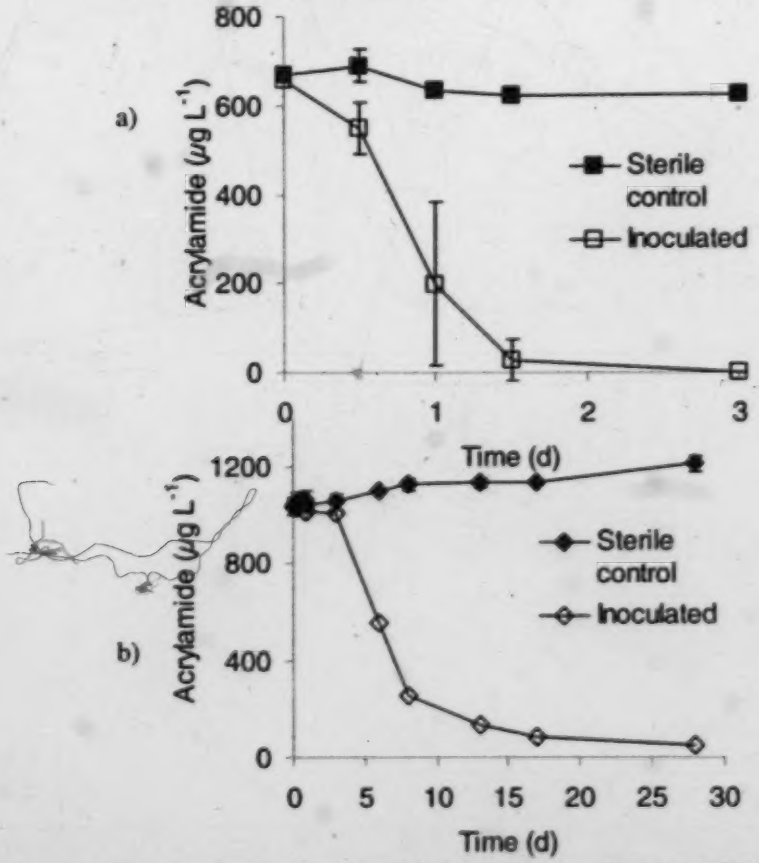

Figure 3.7. Removal of acrylamide by microorganisms in TT in 200 mL shake flask cultures under aerobic conditions. a) B + NP medium, b) 0.8% saline medium.

Although the initial concentrations shown in Figure 3.7 are considerably higher than the low concentrations ($\mu g\ L^{-1}$) expected in TT, the concentration was chosen to allow for quantification of metabolites such as acrylic acid or ammonium. Microorganisms originating from TT were able to degrade acrylamide as a source of carbon and nitrogen under aerobic conditions (Figure 3.7). As expected, when fortified with additional nitrogen and phosphorus, acrylamide degradation proceeded more rapidly than in unsupplemented medium. Cultures inoculated into B + NP medium removed 100% of the added acrylamide in 3 d, whereas cultures in 0.8% saline required nearly 10 times as long to remove 95% of the added acrylamide.

Although acrylamide was not completely removed in saline medium inoculated with TT within the time of this experiment, results from experiments in which acrylamide was spiked directly into TT demonstrate that neither acrylamide nor acrylic acid persist under the aerobic conditions near the surface in TT (Section 2.2.2.1.2). Acrylic acid was removed more quickly than acrylamide (Figure 3.4), which is expected because of the additional deamination step required for acrylamide degradation. When 200 μg acrylamide L^{-1} were added to TT in two replicate experiments, removal was observed in 9 d and 15 d near the surface (Section Section 2.2.2.1.2). Thus, lower acrylamide concentrations do not persist under these simulated environmental conditions.

In the MLSB, low concentrations of nitrogen (average of 3.5 mg L^{-1} as NH_4^+ at 3 m) and phosphorus (0.005 mg L^{-1} as PO_4^{3-} at 3 m) (MacKinnon and Sethi 1993) have been reported. These values originate from depths in the water column (3 m) that would be relatively aerobic; thus, enrichment cultures in 0.8% saline, lacking added nitrogen (other than acrylamide) or phosphorus, would most closely approximate conditions in the release water in the TT deposit. These results correlate with those reported by Semple et al. (2001), who found that shake flask cultures inoculated with MFT degraded 1 mg acrylamide L^{-1} in 1.8 d (B + NP) and 6.3 d (0.8% saline).

Although the concentrations of acrylamide used in this study were considerably lower than concentrations studied by other researchers, complete acrylamide removal occurred in a similar time period. Experiments conducted by Semple et al. (2001) demonstrated that microorganisms in MFT, activated domestic sewage sludge, and garden soil were capable of degrading 50 to 200 mg acrylamide L^{-1} in 4 d. Shanker et

al. (1990) reported that 500 mg acrylamide L^{-1} were completely removed in garden soil and in aerobic cultures inoculated with garden soil in 5 and 6 d, respectively. The total removal of acrylamide by TT-inoculated cultures in supplemented medium in 3 d is comparable to these results. Kumar and Kumar (1998) isolated a *Pseudomonas aeruginosa* strain capable of degrading 2.8 g acrylamide L^{-1} in 40 h, which is more rapid than the rate observed in this study. However, the inoculum used in the Kumar and Kumar (1998) study was from soil regularly exposed to amides, which would acclimate the microorganisms to amides such as acrylamide. Once microorganisms in TT are exposed to acrylamide via PAM treatment over time, acclimation may occur, perhaps leading to increased rates of acrylamide removal.

To verify that acrylamide addition stimulated aerobic bacterial growth, OD_{600} readings were taken on samples taken from aerobic shake flask cultures. However, it was found that there was no difference between killed controls and inoculated cultures in either medium, with values remaining less than 0.01 for the length of the experiment (data not shown). Although no direct correlation can be drawn between acrylamide degradation and bacterial growth based on OD_{600} readings, acrylamide degradation is believed to be biotic, because killed controls demonstrated no acrylamide loss. Likely, the low concentration of acrylamide that was added was not sufficient to cause a discernable increase in OD_{600}.

Acrylamide degradation under aerobic conditions occurs by deamination of acrylamide to acrylic acid (Abdelmagid and Tabatabai 1982), thereby releasing ammonium into the medium (Figure 1.5). If this pathway was followed by the environmental microorganisms in TT, acrylic acid and ammonium would be expected as intermediates in the culture medium. HPLC analysis did not reveal the presence of acrylic acid, nor did colorimetric ammonium analysis detect any ammonium (data not shown). However, in the cultures in 0.8% saline (Figure 3.7a), acrylamide is the only source of added carbon and nitrogen, and so likely acrylic acid and ammonium are consumed rapidly following acrylamide deamination. In cultures with B + NP medium (Figure 3.7b), carbon was limiting, and so acrylic acid was likely consumed readily. Although nitrogen in the form of nitrate was available in B + NP medium, the ammonium released from acrylamide deamination is a readily useable form for

microorganisms (Brown 1980), and so ammonium likely did not persist in the culture medium. Shanker et al. (1990) also failed to detect any ammonium from metabolism of 500 mg acrylamide L^{-1}, and these researchers concluded that the ammonium was likely used as a nitrogen source by microorganisms. If the high concentration of ammonium released from 500 mg acrylamide L^{-1} could not be detected, it is very likely that the amount of potential ammonium available at the much lower initial acrylamide concentration in the aerobic enrichment cultures (Figure 3.7a,b) (less than 1 mg L^{-1}) was readily consumed by aerobic microorganisms.

In summary, acrylamide was found to be readily removed from TT under aerobic and anaerobic incubation conditions at temperatures of 4°C and 22°C by indigenous microbial communities. Based on these experimental results, is seems unlikely that the low concentration of 15 μg acrylamide L^{-1} added from PAM treatment of extraction tailings (planned dosages of 100 to 300 g PAM t^{-1} tailings solids) would persist for any appreciable time (less than 30 d) in the TT deposits formed by the oil sands operations. As a result, this supports the findings that risk of persistence and export of acrylamide is negligible (Kindzierski 2001).

3.1.1.3. Acrylamide biodegradation under sulfate-reducing conditions

Once oxygen has been consumed in deposited oil sands tailings, oxygen-limited conditions will occur. Because of the relatively high sulfate concentration (greater than 200 mg L^{-1}) present in oil sands tailings (Fedorak et al. 2003, Holowenko et al. 2000, Mackinnon and Sethi 1993), sulfate-reducing conditions are expected to predominate.

3.1.1.3.1 Acrylamide biodegradation in high sulfate medium

Enrichment cultures containing sulfate as the dominant terminal electron acceptor (2300 mg L^{-1}) were established to simulate a high sulfate environment. Inocula used were MFT, TT, and mud. Biodegradation by initial enrichment cultures containing both acrylamide and acrylic acid as carbon sources are shown in Figure 3.8.

Results of these initial enrichments with initial acrylamide concentrations of ~ 6 mg L^{-1} showed that both acrylamide and acrylic acid appeared to be biodegraded with a lag phase of less than 7 d (the first sampling time) by the three sources of

microorganisms tested (Figure 3.8). In MFT- and mud-inoculated cultures, acrylic acid was removed before acrylamide, as shown in Figure 3.8a and c. In cultures inoculated with MFT, 74% of the added acrylic acid had been removed by d 14, compared to 54% removal of acrylamide (Figure 3.8a). Mud-inoculated cultures also demonstrated rapid biodegradation of acrylic acid, with 63% removal of acrylic acid by d 7, and total removal by d 14 (Figure 3.8c). However, although 34% of the added acrylamide had been removed from mud-inoculated cultures by d 7, the concentration of acrylamide in these cultures remained constant at ~1.5 mg L^{-1} from d 21 to d 49, at which time the acrylamide had been completely removed. Acrylamide was removed completely from MFT-inoculated cultures in 35 d, while acrylic acid was undetected by d 28 (Figure 3.8c).

In cultures inoculated with TT (Figure 3.8b), acrylic acid and acrylamide were degraded at a rate slower than that of either the MFT- or mud-inoculated cultures. By d 14, only 28% of both the acrylic acid and acrylamide had been removed. Following d 14, the rate more than doubled for both substrates, from a rate of 0.12 mg L^{-1} d^{-1} to 0.28 mg L^{-1} d^{-1} for acrylamide, and from 0.13 mg L^{-1} d^{-1} to 0.27 mg L^{-1} d^{-1} for acrylic acid. However, whereas acrylic acid was completely degraded in cultures inoculated with mud and MFT, ~1.8 mg L^{-1} remained in TT-inoculated cultures after d 49, which represents 27% of the added acrylic acid.

Another set of microcosms was established with only acrylic acid added. Acrylic acid was degraded rapidly by all three inocula in the original enrichments, as shown in Figure 3.9. Mud- and MFT-inoculated cultures demonstrated rapid degradation, with complete removal in 14 and 21 d respectively, whereas TT-inoculated cultures required 70 d for complete removal.

To determine whether sulfate reduction might be coupled to substrate loss, sulfate concentrations were measured, and the results are shown in Table 3.3. Only MFT-inoculated cultures demonstrated significant sulfate loss in cultures containing acrylamide and acrylic acid as carbon sources. Sulfate loss was also expected for TT and mud, because both demonstrated loss of at least one of the substrates, and over half of the other substrate (Figure 3.8b and c). However, this preliminary experiment was not designed to allow for quantification of sulfate, because the amounts of carbon source and

sulfate were not balanced so that stoichiometric sulfate loss could be detected. If the only available sources of carbon were acrylamide and acrylic acid, the decrease in sulfate concentration would be only 29 mg L^{-1}, which is negligible in comparison to the total amount of sulfate added to the medium (~2300 mg L^{-1}). Therefore, sulfate loss due to acrylamide and acrylic acid degradation may not be detected. In cultures containing only acrylic acid, MFT-inoculated cultures were the only cultures that demonstrated significant sulfate loss (Table 3.3), despite substrate loss in the cultures containing the other two inocula. Again, the sulfate required as an electron acceptor (14 mg L^{-1}) for complete removal of 0.1 mM (7 mg L^{-1}) acrylic acid was very low compared to the amount present in the culture medium. Thus, coupling of acrylic acid or acrylamide biodegradation to sulfate reduction could not be demonstrated in these enrichments.

However, a large decrease in the concentration of sulfate was observed in MFT-inoculated cultures containing acrylamide and acrylic acid (Table 3.3). This decrease is more than is expected according to the stoichiometry of acrylamide and acrylic acid consumption, so it is likely that other substrates in MFT in addition to acrylamide and acrylic acid are responsible for the sulfate loss. MacKinnon and Sethi (1993) reported high hydrocarbon concentrations (as bitumen and light hydrocarbons) in MLSB, and postulated that hydrocarbons were likely substrates for microbial growth. Hydrocarbons are susceptible to microbial attack (Atlas and Bartha 1998), and so the residual hydrocarbons remaining in tailings following extraction may be the source of substrates for sulfate reduction. In field studies of the MLSB fine tailings, Holowenko et al. (2000) observed that sulfate loss increased with tailings sediment depth, and also observed a corresponding increase in numbers of SRB. No additional substrates were added to these cultures. Similarly, Fedorak et al. (2003) observed sulfate loss in pore waters of MFT that had been amended with sulfate, but with no additional substrates. Thus, other substrates such as hydrocarbons present in MFT are likely used by SRB.

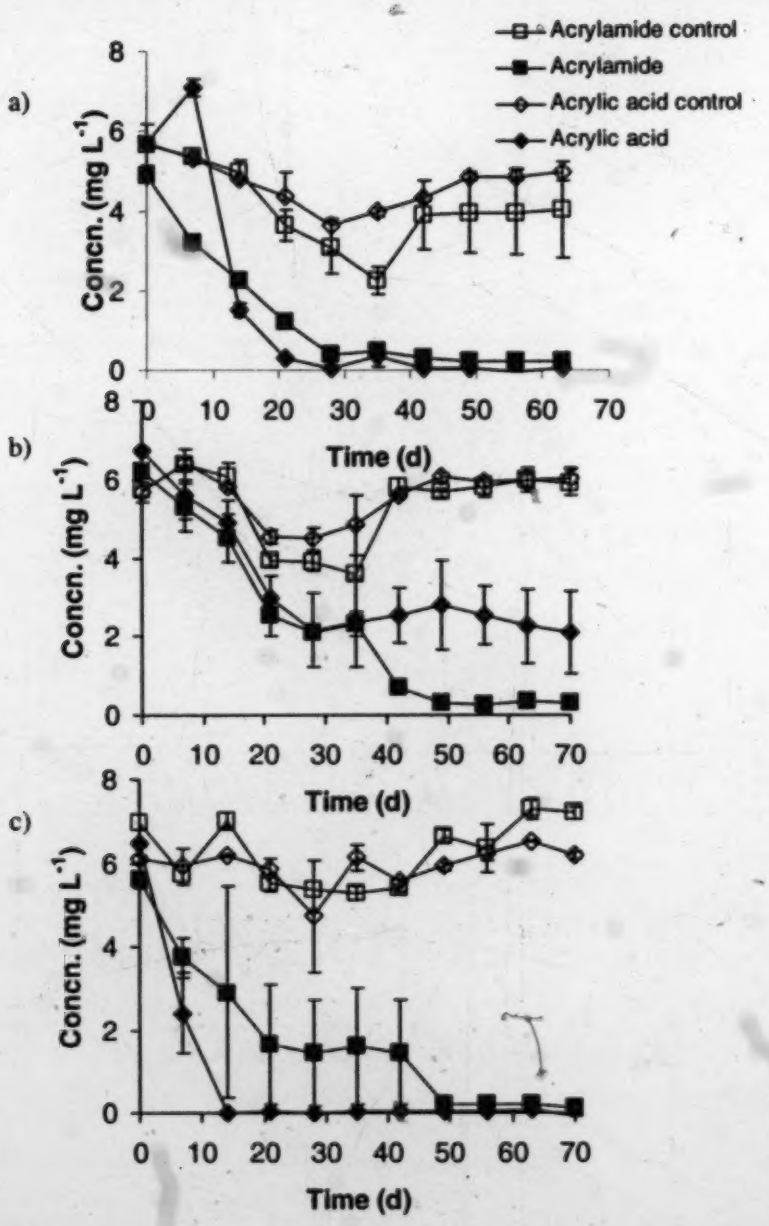

Figure 3.8. Concentrations of acrylamide and acrylic acid in a) MFT-, b) TT-, and c) mud-inoculated microcosms incubated under sulfate-reducing conditions. Error bars represent standard deviation of n=3.

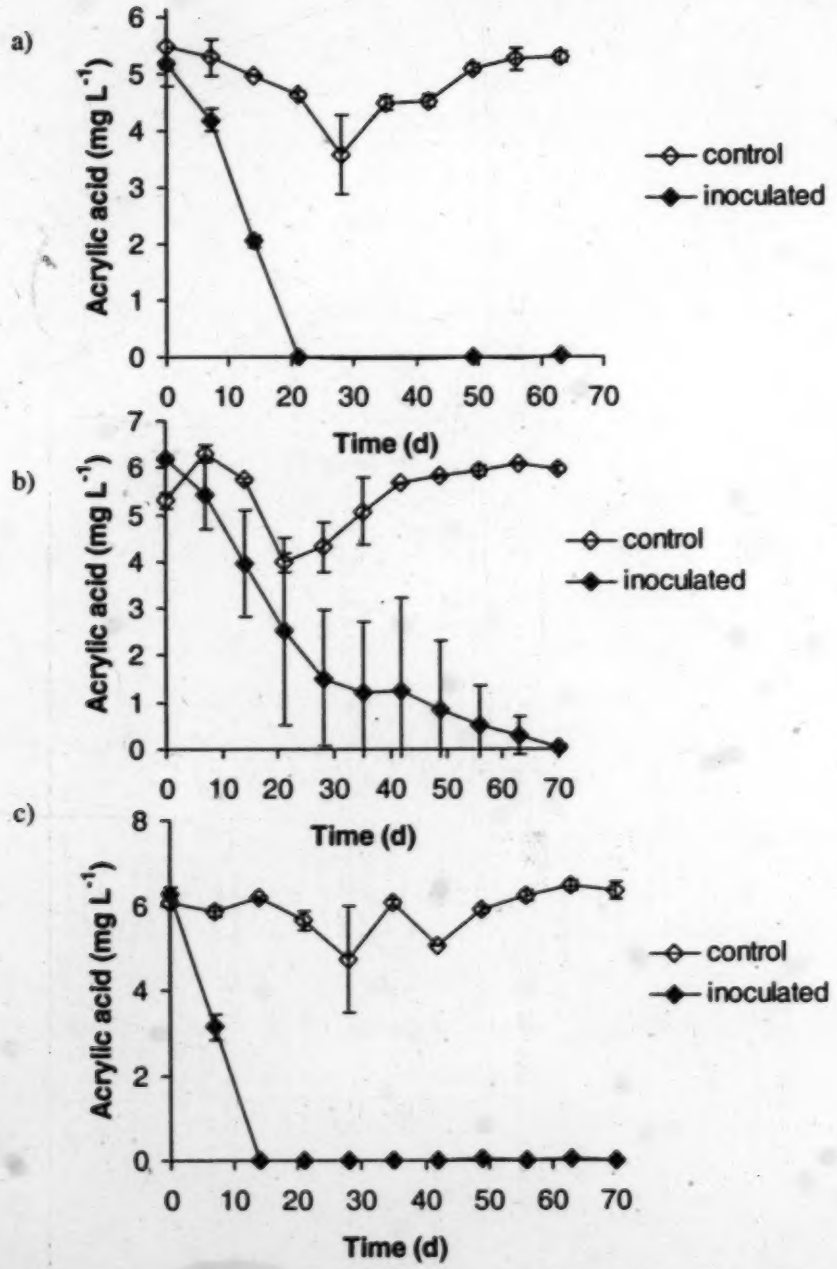

Figure 3.9. Concentrations of acrylic acid in a) MFT-, b) TT-, and c) mud-inoculated microcosms incubated under sulfate-reducing conditions. No acrylamide was added to cultures. Error bars represent standard deviation of n=3.

Table 3.3. Sulfate concentrations in acrylamide and acrylic acid-amended enrichment cultures.

Inoculum	Substrate	Sulfate (mg L^{-1})			
		0 d	21 d	63 d	77 d
MFT	AMD + AA[a]	2280 ± 100[b]	2000 ± 100[c]	1300± 450[c]	ND[d]
TT	AMD + AA	2490 ± 30	2450 ± 30	ND	2400 ± 170
Mud	AMD + AA	2480 ± 50	2400 ± 30	ND	2390 ± 60
MFT	AA	2200 ± 80	2200 ± 140	1600 ± 70[c]	ND
TT	AA	2440 ± 30	2390 ± 60	ND	2400 ± 40

[a] AMD – acrylamide; AA – acrylic acid
[b] standard deviation of n=3
[c] mean significantly different from time 0 mean ($p<0.05$)
[d] ND not determined

3.1.1.3.2 Acrylamide biodegradation in low sulfate medium

Serial transfers into low sulfate medium (290 mg L^{-1}) containing a higher concentration of substrate (~70 mg L^{-1}) were undertaken to attempt to demonstrate sulfate loss coupled to acrylamide and acrylic acid removal. In cultures containing only acrylamide, this tenfold higher concentration may have been toxic, because little acrylamide was removed by any inoculum during the experiment, as shown in Figure 3.10. MFT-inoculated cultures did not demonstrate significantly different concentrations of acrylamide in active cultures and heat-killed controls (Figure 3.10a). Similarly, little degradation was observed in cultures inoculated with TT, as shown in Figure 3.10.

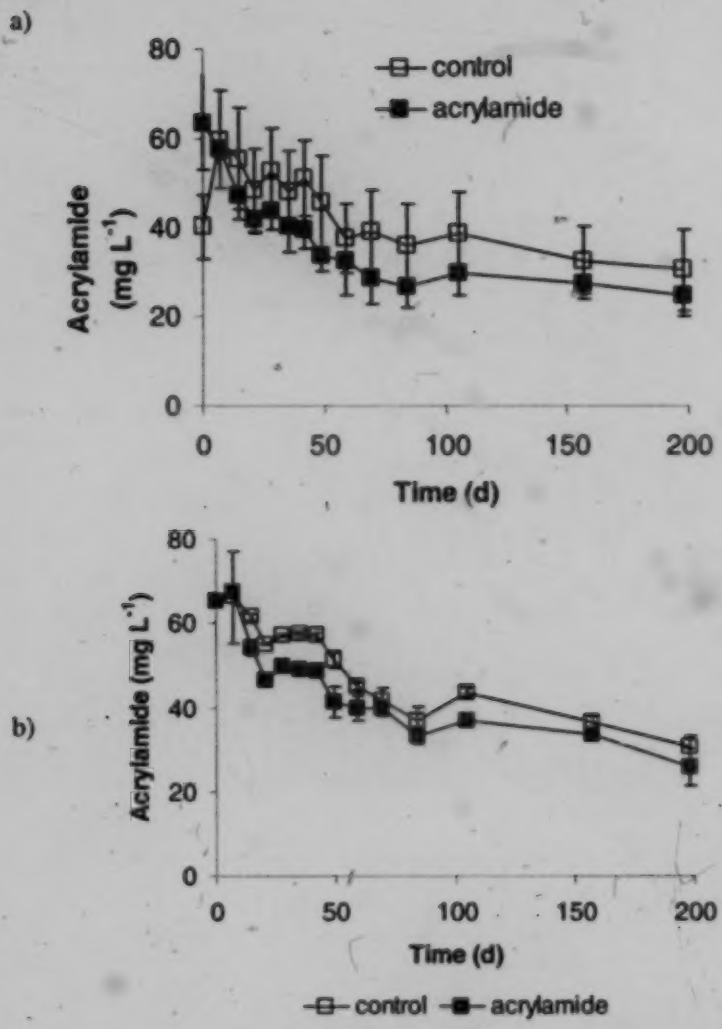

Figure 3.10. Concentrations of acrylamide in enrichment cultures after three serial transfers containing a) MFT and b) TT as inocula incubated under sulfate-reducing conditions in medium that contained 290 mg sulfate L^{-1}. Error bars represent standard deviation of n=3.

Although acrylamide alone appeared toxic to the environmental consortia as demonstrated by the persistence of acrylamide (Figure 3.10), when acrylic acid and acrylamide were added in combination, degradation of both compounds was observed (Figure 3.11). In MFT-inoculated cultures, acrylamide removal began before degradation of acrylic acid (Figure 3.11a). This result was unexpected, because acrylamide degradation involves an additional deamination step over degradation of only acrylic acid. A lag time of less than 7 d was observed, with acrylamide removed at a rate of 2.1 mg L^{-1} d^{-1} until d 21, after which time the rate slowed. In fact, from d 21 to d 42, only 5 mg acrylamide L^{-1} were removed. Following d 42, the rate increased to 1 mg L^{-1} d^{-1}, with complete removal occurring by d 157.

Acrylic acid degradation also occurred in MFT-inoculated cultures (Figure 3.11a). Two replicates demonstrated a lag phase of less than 42 d, whereas one replicate demonstrated a lag phase of an additional week. By d 49, only 1 mg L^{-1} remained, with total removal by d 59 (Figure 3.11a). This inoculum was the only one of the three tested in which degradation of both substrates occurred. From the results of the experiments with only acrylic acid (Figure 3.9) and with both acrylic acid and acrylamide (Figure 3.11), it is clear that when acrylic acid is present alone, degradation occurs more rapidly.

TT-inoculated cultures demonstrated loss of acrylamide initially, as shown in Figure 3.11b. Of the three replicates, two demonstrated little removal at later time points, so the results from these two replicates were averaged and plotted. The results from the other replicate were plotted separately. In the two averaged replicates, the initial rate of acrylamide removal was quite rapid, with 2.3 mg L^{-1} d^{-1} from d 0 to d 21, which was similar to the rate of 2.1 mg L^{-1} d^{-1} shown by MFT-inoculated cultures. From d 21 to d 42, the rate slowed, with only 6 mg L^{-1} removed in 21 d, which mirrored the trend observed in MFT-inoculated cultures. Following d 42, the concentration decreased from 72 mg L^{-1} to 58 mg L^{-1} by d 49, with only an additional 11 mg L^{-1} removed in the following 149 d. In the third replicate, degradation rates were similar to the previous two, with one important difference. In this replicate, only 4.2 mg L^{-1} acrylamide remained by d 198, compared to 47 mg L^{-1} remaining in the other replicates. Surprisingly, no acrylic acid loss was observed, which differed from the results of the

MFT-inoculated cultures. This may result from acrylic acid production during acrylamide degradation.

In mud-inoculated cultures, very little acrylamide was removed, with only 28 mg L^{-1} (30% of the total added) removed in 69 d (Figure 3.11c). Only two of the three replicates demonstrated acrylic acid removal, as observed in Figure 3.11c. After a lag phase of about 21 d, acrylic acid was removed at a rate of 3 mg L^{-1} d^{-1}, with complete removal by d 49. The third replicate demonstrated no acrylic acid loss.

To ensure that SRB were present in these cultures after three serial transfers, samples of each culture (MFT-, TT-, and mud-inoculated) were transferred into Butlin's medium as described in Section 2.1.16. All three inocula demonstrated a black precipitate on iron nails after 30 d incubation, indicating that SRB were present and active (data not shown).

Although the conditions maintained in these experiments were intended to select for SRB, no sulfate loss was detected in any of the cultures that displayed substrate loss (data not shown). Methane analyses verified that there is no methanogenic activity in these cultures (data not shown). Thus, the coupling of acrylamide or acrylic acid biodegradation to sulfate reduction could not be demonstrated.

3.1.1.3.3 Acrylamide biodegradation with molybdate added as an inhibitor of SRB

Verification of sulfate reduction coupled to acrylamide degradation was sought, so molybdate was added to a series of cultures to inhibit SRB (Banat et al. 1983). If SRB are responsible for acrylamide biodegradation, no acrylamide degradation should occur in cultures containing molybdate. The results of this experiment are shown in Figure 3.12. Acrylamide was degraded in cultures containing only acrylamide, as well as in cultures containing acrylamide and sulfate added as a terminal electron acceptor (Figure 3.12a). The sulfate concentration decreased in the cultures with added sulfate and acrylamide (Figure 3.12b), which may indicate the activity of SRB. However, the loss of acrylamide in cultures with no added sulfate (Figure 3.12a) confounds this conclusion, because the sulfate concentration in these cultures was below detection (10 mg L^{-1}). As well, sulfate loss was observed in cultures containing no added acrylamide, which raises

the possibility that other native carbon sources such as residual bitumen, and not acrylamide, may be the substrate of SRB in these cultures.

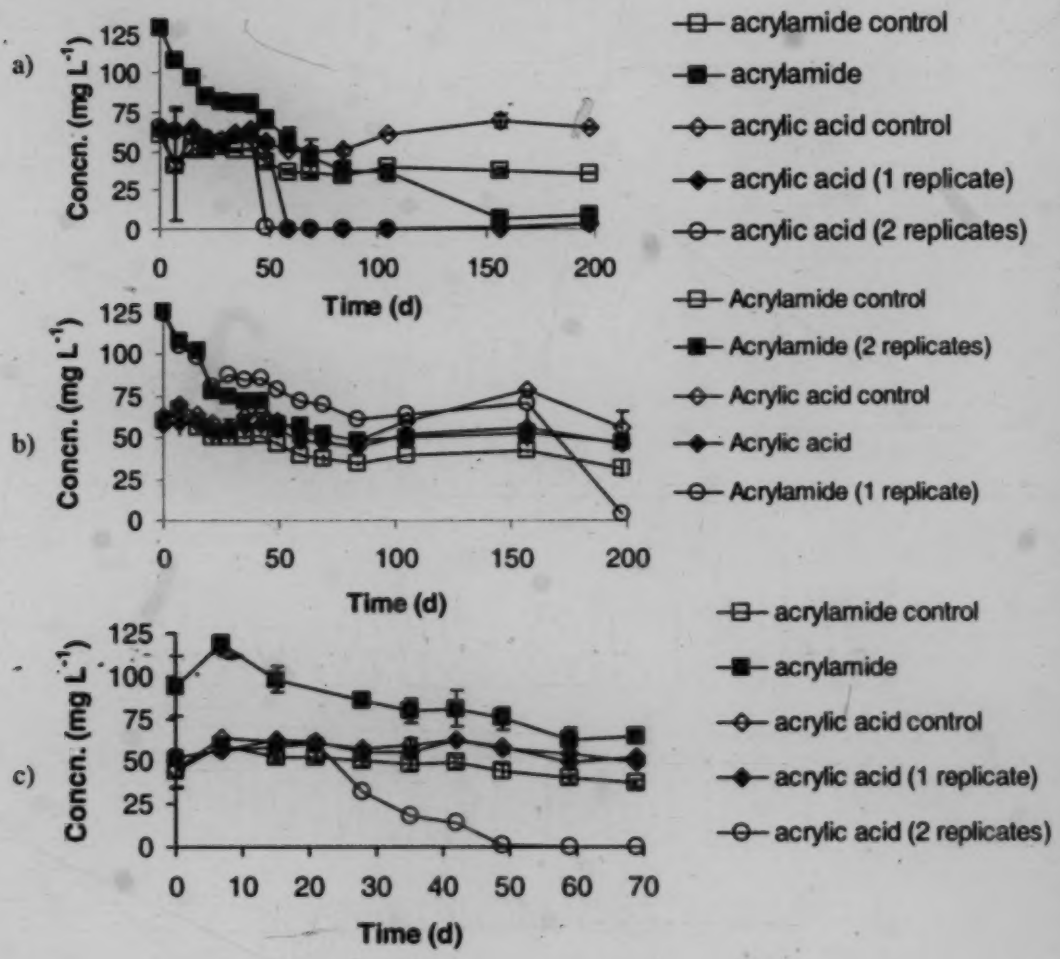

Figure 3.11. Concentrations of acrylamide and acrylic acid in a) MFT, b) TT, and c) mud-inoculated microcosms incubated under sulfate-reducing conditions after three serial transfers. Replicates that have been plotted separately are indicated in brackets; averaged values were plotted for those with two replicates. Error bars represent standard deviation of n=3.

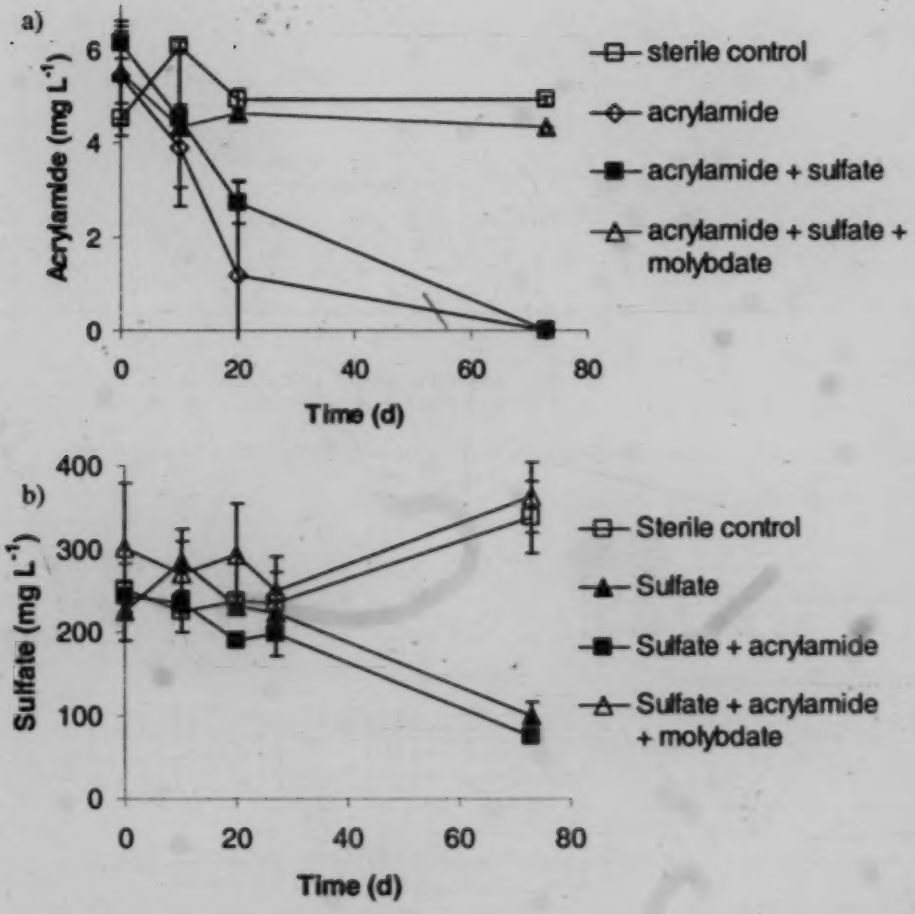

Figure 3.12. Activity of SRB in MFT when supplemented with acrylamide as a carbon source (original enrichment). a) acrylamide concentrations, b) sulfate concentrations. Error bars represent ± 1 standard deviation of n=3.

Although cultures were established under conditions designed to select for SRB, little evidence of SRB activity (sulfate loss) was observed in relation to substrate loss. Although sulfate loss was observed in cultures containing acrylamide and sulfate (Figure 3.12b), sulfate was removed in cultures with no added acrylamide, so coupling of

acrylamide degradation to sulfate loss could not be confirmed. One possible explanation is that added sulfate originating from the inoculum in the original enrichment may have masked any sulfate loss caused by SRB activity. Based on an average sulfate concentration of 160 mg L^{-1} in oil sands tailings (Holowenko et al. 2000), the amount of sulfate contributed by the inoculum is approximately 50 mg L^{-1} sulfate, which calculates to one-fifth of the sulfate added to the medium (acrylamide or acrylic acid individually), or less than that, in the case of cultures containing both substrates. By transfer 3, this added sulfate would account for only 1/500 of the original concentration, which is a negligible amount. Therefore, even with this additional sulfate in the medium, some sulfate loss should be detected if SRB were responsible for acrylamide or acrylic acid removal.

The presence of alternative terminal electron acceptors (TEAs) that would allow for a competitive advantage might explain this loss, but neither identification nor quantification of TEAs such as nitrate, iron, or manganese was performed. However, the only source of such TEAs would be the tailings inoculum, because the medium contains only trace amounts of iron and molybdate. According to MacKinnon and Sethi (1993), water in MLSB contained low concentrations of dissolved iron (less than 0.10 mg L^{-1}), which would be further diluted upon inoculation into culture medium. Also, the iron in tailings water will be in the reduced form (Fe^{2+}), so with a low iron and its oxidation state, the possibility of it acting as a TEA is unlikely.

Degradation of acrylamide or acrylic acid by aerobic or methanogenic microorganisms was unlikely in these cultures, because all cultures remained reduced, as indicated by the colorless state of the resazurin indicator (therefore no O_2 was present), and no methane was detected throughout the experiment. One remaining possibility is that heterotrophic bacteria present in the oil sands tailings are capable of acrylamide and acrylic acid removal. Abdelmagid and Tabatabai (1982) reported hydrolysis of 500 mg L^{-1} acrylamide to acrylic acid in waterlogged soils held under a nitrogen atmosphere. After 21 d, between 76% and 93% of the added acrylamide-N was recovered, with the recovery depending on soil physical and chemical properties (Abdelmagid and Tabatabai 1982). Brown (1980) found that 5 mg L^{-1} acrylamide was degraded in 21 d when river water samples were held under nitrogen in the dark, compared to removal in 15 d when

incubated under nitrogen and exposed to light. Neither study reported any identification of the anaerobic community responsible for the acrylamide degradation. Although acrylamide and acrylic acid degradation by SRB could not be conclusively demonstrated in oil sands tailings, the results of these experiments demonstrate that these compounds are removed under anaerobic conditions. No other studies in the literature have attempted to study biodegradation of these compounds under sulfate-reducing conditions.

3.1.1.4. Acrylamide biodegradation under methanogenic conditions

3.1.1.4.1 Enrichment cultures

TT are initially aerobic during production and deposition, but following deposition, oxygen replenishment from the atmosphere will cease, causing the deposit to become anoxic. If oxygen is depleted prior to complete acrylamide removal (30 d with no added nutrients, Figure 3.7b), anaerobic conditions will occur. Once sulfate is depleted as a terminal electron acceptor in oil sands tailings, methanogenic conditions are expected to predominate (Holowenko et al. 2000, Fedorak et al. 2002). The time required to achieve these conditions will vary. Methanogens are present in oil sands tailings, with reported concentrations of 10^5-10^6 MPN g^{-1} of tailings (Holowenko et al. 2000). The ability of methanogenic consortia to degrade low concentrations (less than 20 $\mu g\ L^{-1}$) of acrylamide is of interest, because these low concentrations are expected in TT deposits. Although the actual concentration of residual acrylamide in PAM is expected to be in the $\mu g\ L^{-1}$ range, such a low concentration would not be sufficient to detect whether acrylamide could enhance methanogenesis. Thus, higher concentrations (7 mg L^{-1} and 70 mg L^{-1}) were chosen for preliminary study.

In enrichment microcosms, the lower concentration (approximately 7 mg L^{-1}) of acrylamide was readily degraded by microcosms inoculated with MFT and with anaerobic sewage sludge, as shown in Figure 3.13a and c respectively. Removal in MFT-inoculated cultures (Figure 3.11a) occurred rapidly, with 59% of the added acrylamide depleted by d 19. Following d 19, the rate of removal slowed, but all of the added acrylamide was depleted by d 90. In sewage sludge-inoculated cultures (Figure 3.13c), acrylamide was removed very quickly, with complete loss in just 10 d. Acrylamide

removal in TT-inoculated cultures was much slower. After 291 d incubation, TT-inoculated cultures supplemented with 7 mg acrylamide L^{-1} (Figure 3.13b) demonstrated significant substrate loss, although approximately 25% of the added acrylamide remained.

Interestingly, a peak with the same HPLC retention time as acrylic acid was detected in all three replicates of cultures with added acrylamide inoculated with MFT (Figure 3.13a). When quantified, an increase in the concentration of this metabolite was found to closely follow the loss of acrylamide in the culture, as shown in Figure 3.13a. By d 10, approximately half of the added acrylamide (3.2 mg L^{-1}, or 0.045 mM) had been removed, with corresponding appearance of 2.6 mg L^{-1} (0.037 mM) of the metabolite believed to be acrylic acid. Attempts were made to verify that this product is acrylic acid using three different derivatization methods coupled with HPLC, GC, or GC-MS, but were unsuccessful, presumably because of the low molecular weight of acrylic acid which made separation from peaks originating from derivatizing reagents difficult.

High background methane production made detection of methane enhancement from acrylamide biodegradation difficult during this initial enrichment, as seen in Figure 3.14. Microcosms inoculated with sewage sludge demonstrated no difference between cultures supplemented with acrylamide and those containing no added carbon source (Figure 3.14b). In MFT-inoculated cultures, cultures that received no acrylamide appeared to have a significantly higher level of methane than did cultures containing either concentration of acrylamide, as shown in Figure 3.14a. TT-inoculated cultures containing ~ 5 mg acrylamide L^{-1} produced no methane, whereas cultures lacking acrylamide produced ~3% (vol.) methane in 291 d (data not shown).

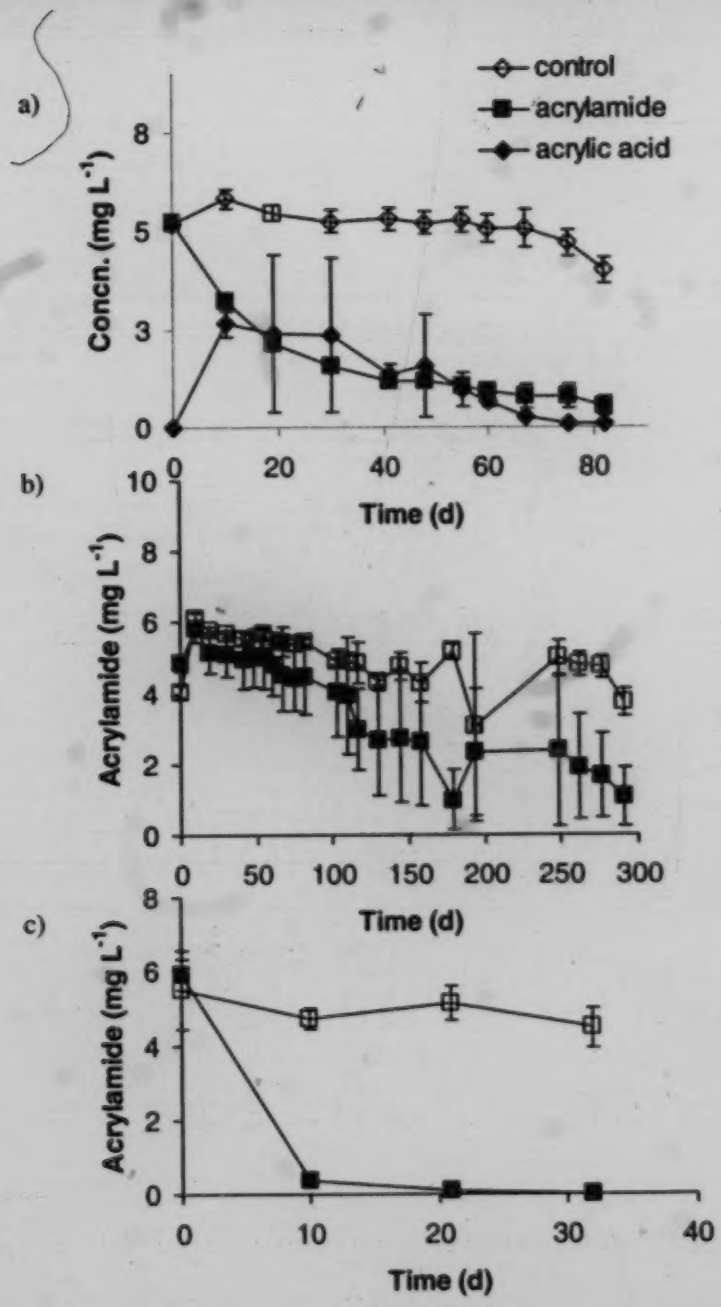

Figure 3.13. Concentrations of acrylamide in microcosms inoculated with a) MFT, b) TT, and c) sewage sludge and incubated under methanogenic conditions. Error bars represent standard deviation of n=3.

Once acrylamide had been removed from microcosms inoculated with MFT and sewage sludge, transfers into fresh medium containing 7 mg acrylamide L^{-1} were done. Transfers were performed to dilute any remaining carbon originating from inocula to allow for detection of methane production originating from acrylamide carbon. However, following these transfers, no significant acrylamide loss was detected in cultures inoculated with either environmental source of microorganisms. Methane production was not significantly different from the unsupplemented control in sewage sludge-inoculated cultures, and was insignificant in MFT-containing cultures.

Based on these results, it appears that acrylamide was removed from microcosms under methanogenic conditions, although enhanced methanogenesis could not be demonstrated in these enrichments. Conversely, the methanogenic consortia in the two oil sands inocula tested appeared inhibited by the relatively low concentrations of acrylamide used. Sewage sludge-containing cultures maintained their methanogenic activity in the presence of relatively low and high concentrations of acrylamide (Figure 3.14b), so sewage sludge was chosen to conduct an inhibition assay to determine which concentrations of acrylamide may be inhibitory to acetate-utilizing methanogens.

3.1.1.4.2 Inhibition studies with acrylamide in methanogenic consortia

To assess toxicity effects of acrylamide on methanogenesis, sewage sludge-inoculated cultures were provided with acetate as a readily-used carbon and energy source, and challenged with various concentrations of acrylamide (7 to 710 mg L^{-1}). The concentrations of methane produced were compared to the concentrations of methane in cultures with no added acrylamide.

There was no significant inhibition of methanogenesis in cultures supplemented with 7 or 40 mg acrylamide L^{-1}, as the methane level was similar to that of the control containing only acetate (Figure 3.15). The final methane concentrations in the cultures with the three lower concentrations of acrylamide were approximately the same, with values of 38%, 39%, and 41% in cultures containing 7, 40, and 70 mg acrylamide L^{-1}. Acrylamide was removed completely in these cultures in 29, 52, and 116 d respectively (data not shown).

Initially, acrylamide at nominal concentrations of 70, 360, and 710 mg L^{-1} significantly inhibited methanogenesis in cultures inoculated with sewage sludge, as shown in Figure 3.15 ($p<0.05$). However, by d 83, methane production increased in cultures containing the two highest concentrations of acrylamide. In cultures supplemented with 360 or 710 mg acrylamide L^{-1}, the methane level increased from ~12% and 8% to maximum values of 40% (vol.) and 46% (vol.) respectively by d 156.

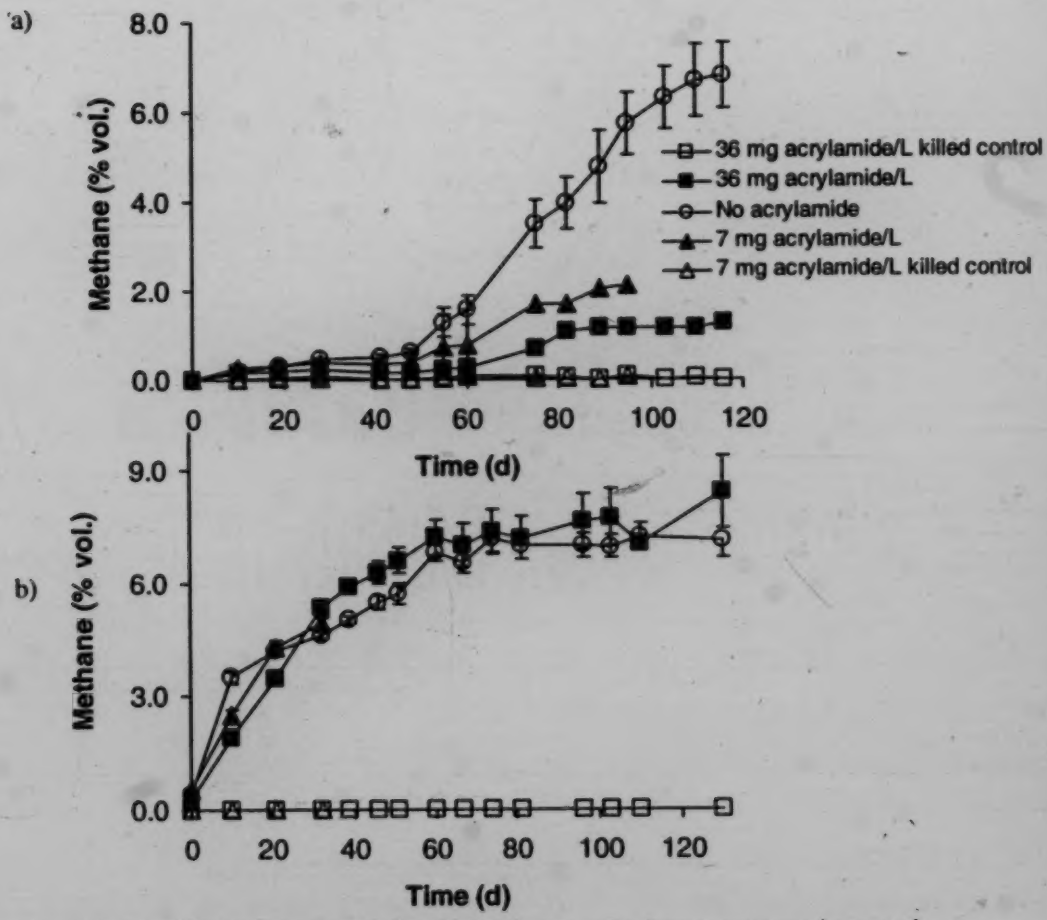

Figure 3.14. Methane concentration in headspace of microcosms supplemented with acrylamide and inoculated with a) MFT, and b) sewage sludge. Error bars represent standard deviation of n=3.

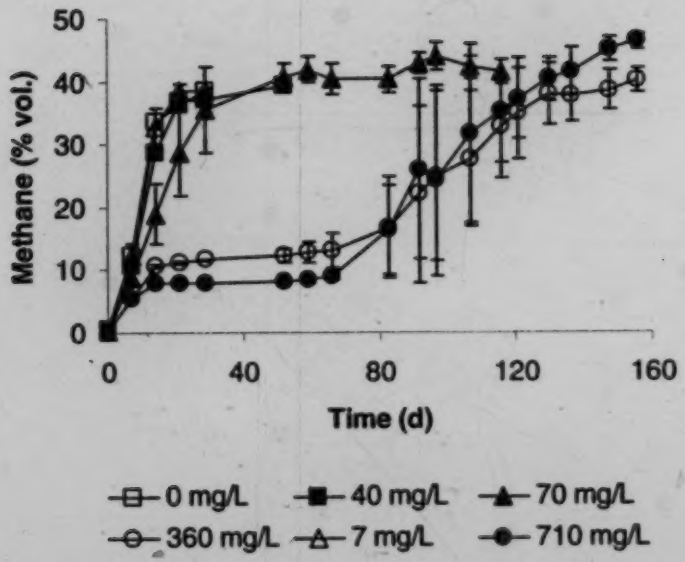

Figure 3.15. Effect of acrylamide on methanogenesis by sewage sludge-inoculated microcosms. Error bars represent standard deviation of n=3.

Acrylamide removal was observed in cultures supplemented with each concentration of acrylamide. Cultures initially containing 7 mg acrylamide L^{-1} removed all measurable acrylamide in 29 d, whereas cultures initially containing 40 and 70 mg acrylamide L^{-1} took considerably longer for acrylamide removal, with 16% and 26% of the added acrylamide remaining after 29 d and 116 d, respectively (data not shown). In these cultures, although there did not appear to be a defined lag phase, the rates of acrylamide removal were quite slow over the course of the experiment. In contrast, cultures initially containing 360 and 710 mg acrylamide L^{-1} demonstrated a 14 d lag phase before acrylamide degradation began (data not shown). Once degradation began, it progressed rapidly, with complete removal by d 66. Figure 3.15 shows that methane production increased markedly after d 66 in the cultures that initially contained 360 or 710 mg acrylamide L^{-1}. Thus enhanced methane production occurred only after acrylamide was biodegraded completely in these cultures. The results in Figure 3.15 clearly demonstrate that high concentrations of acrylamide inhibit methanogenesis.

However, after sufficient incubation time, the inhibition is overcome and methane is produced in batch cultures.

3.1.1.4.3 Attempts to demonstrate enhanced methanogenesis from acrylamide biodegradation

Unacclimated methanogenic enrichment cultures did not demonstrate enhanced methanogenesis in the presence of acrylamide (Section 2.2.2.4.1). However, the sewage-sludge-inoculated cultures established in the inhibition study (Section 3.1.1.4.2) provided a consortium acclimated to various concentrations of acrylamide, and so this consortium was used in an attempt to demonstrate that acrylamide biodegradation could yield methane production. To do so, once all acrylamide from the original addition had been degraded, the headspaces of the cultures were flushed with oxygen-free gas to reduce the methane concentration to nil, and acrylamide was added to restore original concentrations used in the inhibition study. Methane and acrylamide concentrations were analyzed to determine if acrylamide enhanced methanogenesis.

Following addition of acrylamide and headspace flushing, methane production proceeded immediately in cultures initially containing 7 mg acrylamide L^{-1}, but this methane concentration was not significantly different from the acrylamide-free control (Figure 3.16). An initial acrylamide concentration of 40 mg L^{-1} produced similar results. An initial acrylamide concentration of greater than 70 mg L^{-1} caused significant inhibition of methane production during this experiment.

Of all the concentrations tested, the results of the two highest concentrations (360 and 710 mg L^{-1}) were the most dramatic. After supplementing with acrylamide, lag periods of about 41 d (360 mg L^{-1}) and 48 d (710 mg L^{-1}) were observed in these cultures (Figure 3.16). In cultures initially containing 360 mg acrylamide L^{-1}, methane production increased rapidly to a final value of 10±8% (vol.) reached at d 114. The large standard deviation was caused by two of the replicates demonstrating methane concentrations of 16% (vol.) and 13% (vol.) on d 114, giving an average methane concentration of 14%, (vol.) while the final replicate demonstrating a relatively constant methane production of 1% (vol.). The mean of the two replicates demonstrating higher

methanogenesis (14% vol.) is higher than the control mean of 6±0.8% (vol.), showing that acrylamide at this concentration leads to increased methanogenesis.

The same situation occurred in cultures supplemented with an initial acrylamide concentration of 710 mg L^{-1}. In these cultures, methane production increased following d 48, eventually surpassing the control value of 6% (vol.) by d 114, with a value of 20±7% (vol.). Again, the large standard deviation is explained by two of the three replicates displaying significantly higher values than the third. For instance, at d 114, two replicates had concentrations of 24% (vol.) each, while the third remained considerable lower, at 12% (vol.). Despite the high standard deviation, the mean value of 20% is significantly higher than the control value of 6±0.8% (vol.), demonstrating that this acrylamide concentration causes a significant increase in the amount of methane produced in acclimated sewage sludge-inoculated cultures.

Because of time constraints, this experiment had to be terminated at d 114. However, methane production in the cultures with 360 or 710 mg acrylamide L^{-1} had not demonstrated any leveling by the end of this experiment, and so more methane production is likely. At d 114, the amounts of methane observed in cultures containing 360 and 710 mg acrylamide L^{-1} represented approximately 62% and 67% of the total methane expected by Buswell's equation from these concentrations of acrylamide (Equation 2.1).

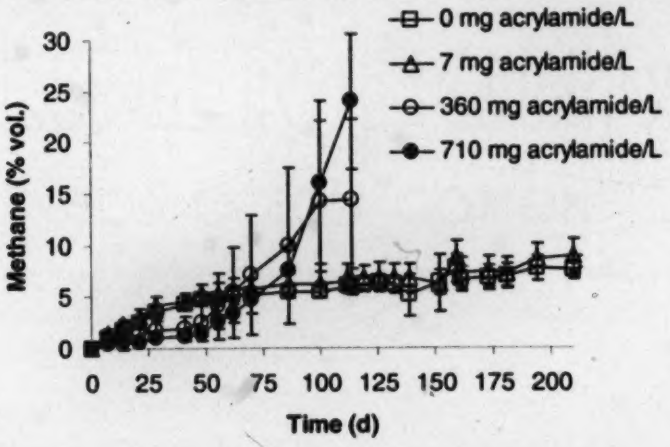

Figure 3.16. Methane concentration in headspace of acclimated sewage sludge-inoculated cultures supplemented with various concentrations of acrylamide.

Acrylamide removal after re-addition of acrylamide is shown in Figure 3.17. The results from the three lowest concentrations are shown in Figure 3.17a, whereas the results from the two higher concentrations are shown in Figure 3.17b. The lowest concentration (7 mg L^{-1}) was removed quickly, with 90% removed in 14 d. After d 14, the rate in two of the three replicates was approximately the same, with complete removal in these two by d 64. The third replicate had 120 μg acrylamide L^{-1} remaining on d 72. On d 78, acrylamide was added to all three replicates to bring the concentration back to 7 mg L^{-1}, but the headspace was not flushed so that methane production resulting from acrylamide addition might be observed. However, the methane production in these acrylamide-supplemented cultures was not significantly different from the amounts in the controls with no added acrylamide. This was also the case in cultures initially containing 40 mg acrylamide L^{-1}, although results may have been different had all the acrylamide been removed. As of d 139, the last sampling time, greater than 25 mg L^{-1} of the added acrylamide had been used in these cultures. Interestingly, the cultures initially containing 70 mg acrylamide L^{-1} produced an average of 4.6% (vol.) methane by d 135, which is significantly lower than the control. By the last sampling day (d 93), 63% of the added acrylamide had been consumed, with no increase in methane production. As was observed in cultures with the highest acrylamide concentrations prior to flushing of the headspaces, methane production did not increase significantly until after acrylamide had been removed. As seen in Figure 3.17b, acrylamide removal was complete in the higher concentrations by d 35 (710 mg L^{-1}), and had reached ~3% of the total added in the 360 mg L^{-1} cultures by the last sampling time (d 41).

The higher initial concentrations of acrylamide allowed for the accumulation of a metabolite with the same retention time as acrylic acid (Figure 3.17b). When quantified, the concentration of the metabolite was lower than the concentration expected resulting from acrylamide deamination. For instance, in one replicate initially contained 846 mg acrylamide L^{-1} at d 0. At d 14, the concentrations of acrylamide and the unidentified metabolite were 560 mg L^{-1} and 47 mg L^{-1} respectively. Thus, this low concentration of metabolite is consistent with acrylic acid production from acrylamide. The "acrylic acid" concentration did not appear to follow any pattern. A low concentration was present at d 0, which is likely caused by acrylic acid left in the cultures following the inhibition

study. This concentration increased to d 7, remained steady until d 21, at which time the concentration decreased to 0 by d 35.

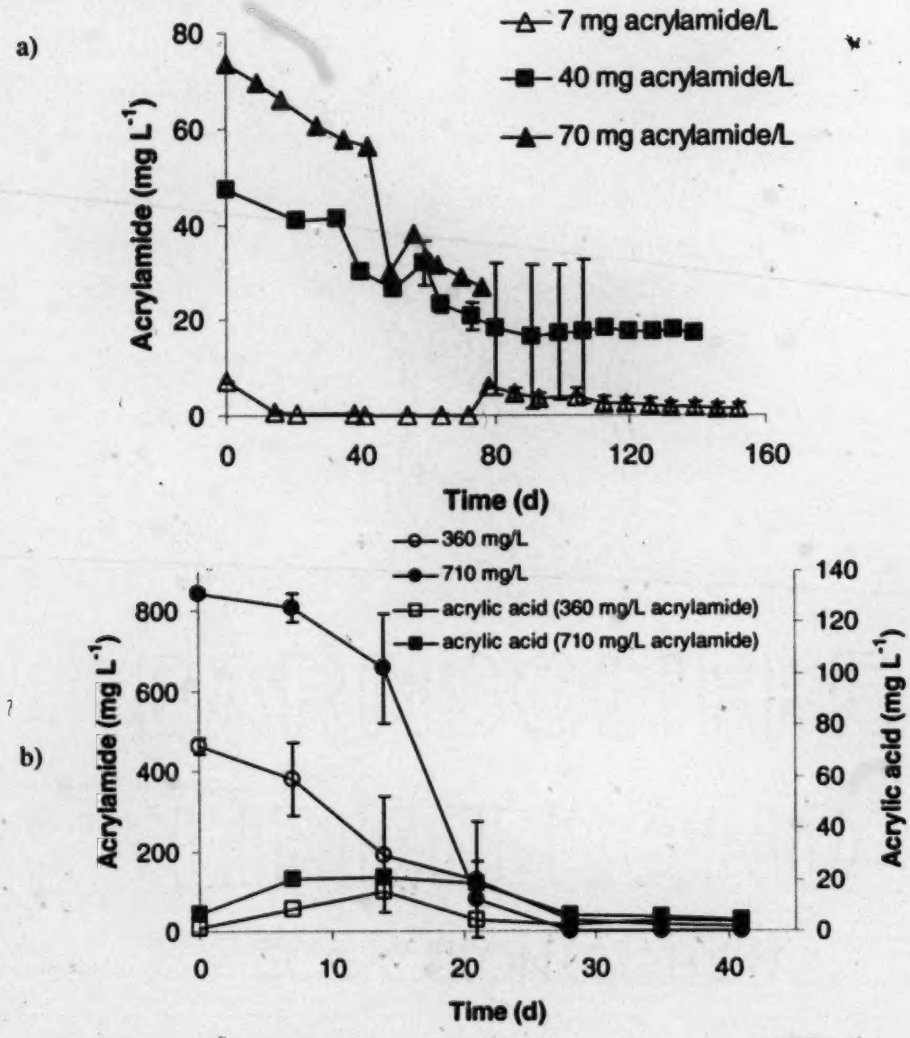

Figure 3.17. Acrylamide degradation by sewage sludge-inoculated cultures under methanogenic conditions after headspace flushing. a) the three lowest acrylamide concentrations used, b) the two highest acrylamide concentrations used. The intended acrylamide concentrations after re-supplementation are shown in the legends; the metabolite suspected to be acrylic acid is also shown.

Acrylamide demonstrated a reversible inhibitory effect on methanogenic microcosms inoculated with sewage (Figure 3.15). Higher concentrations (360 and 710 mg acrylamide L^{-1}) inhibited methane production significantly, but once cultures became acclimated, methane production increased to surpass the concentration in acrylamide-free viable controls. If the final methane concentrations in these two cultures are compared to the calculated theoretical levels (Table 3.4), it can be seen that actual methane amounts in cultures containing initial concentrations of 460 and 850 mg acrylamide L^{-1} (10% and 20%) remained below the calculated amounts of methane (20.9% and 32.5%) at the end of the experiment. However, methane production had not yet reached a plateau in these cultures after 114 d of incubation, so the theoretical values may be reached if monitoring had been continued. Culture containing the lowest acrylamide concentrations (7, 40, and 70 mg L^{-1}) displayed no enhanced methanogenesis, although over half of the original acrylamide added had been consumed in these cultures by the end of sampling (d 173, d 160, and d 97, respectively) (Figure 3.17).

Enrichment cultures inoculated with oil sands tailings inocula degraded acrylamide, albeit at different rates. MFT degraded 100% of 5 mg acrylamide L^{-1} in just over 80 d, whereas TT-inoculated cultures only removed 75% by d 291 (Figure 3.13). Detectable amounts of a metabolite believed to be acrylic acid were observed in MFT-inoculated cultures, although verification of its identity was unsuccessful. This metabolite accumulated briefly, then was consumed in these microcosms.

Although no other studies on acrylamide degradation under methanogenic conditions have been reported, acrylic acid is degraded anaerobically, with initial inhibition of gas production at higher concentrations. When challenged with concentrations of 250 to 750 mg acrylic acid L^{-1}, gas production in acetate-enriched methanogenic mixed cultures was inhibited from 9 to 16 d, after which gas production resumed (Qu and Bhattacharya 1996). Demirer and Speece (1998a) exposed unacclimated acetate-enriched pure cultures of *Methanosarcina* sp. to concentrations of acrylic acid ranging from 20 to 600 mg L^{-1}, and found that all concentrations above 20 mg L^{-1} depressed gas production with no recovery of gas production over the course of the 14-d experiment. Schonberg et al. (1997) also conducted toxicity assays using an

unacclimated culture from an anaerobic digester, and found that inhibition of gas production corresponded to increased acrylic acid concentrations.

Table 3.4. Theoretical amount of methane calculated from Buswell's equation from different acrylamide concentrations in an acrylamide toxicity assay with 85 mL of liquid in 158-mL serum bottles.

Initial acrylamide concentration ($mg\ L^{-1}$)	Initial acrylamide concentration (mmol)	Methane yield from acrylamide (mmol)	Calculated methane yield (mL at STP)	Potential methane in headspace (% vol.)[a]
7	0.01	0.01	0.28	0.4
50	0.06	0.09	1.9	2.6
70	0.09	0.13	3.0	4.0
460	0.56	0.83	18.7	20.9
850	1.0	1.5	34.0	32.5

[a] calculated according to method shown in Section 2.1.19, using Equation 2.1, assuming a headspace volume of 70.5 mL.

Intermediate metabolites of acrylic acid degradation included propionate and acetate (Demirer and Speece 1998a, Qu and Bhattacharya 1996, Schonberg et al. 1997), indicating that acrylic acid metabolism likely follows the pathway depicted in Figure 1.5. However, it is unlikely that acrylic acid is degraded by methanogens, because bromoethanesulfonic acid, an inhibitor of methanogenesis, did not stop acrylic acid degradation in acetate-enriched methanogenic mixed cultures, leading these researchers to conclude that acetogens are responsible for acrylic acid degradation (Qu and Bhattacharya 1996).

The hypothesis that non-methanogens present in a consortium may be responsible for acrylic acid degradation may explain the observation that methane production in sewage-inoculated cultures provided with 360 and 710 mg acrylamide L^{-1} in the toxicity assay did not begin until after all the acrylamide had been degraded. If other

microorganisms are required to deaminate acrylamide and degrade acrylic acid to a more readily-usable substrate for methanogenesis, methane production would be delayed.

3.1.1.5. Kinetics of acrylamide biodegradation

The studies discussed in Section 3.1.1 have provided data from which kinetic constants can be calculated using the method outlined in Section 2.1.19. Table 3.5 gives the kinetic values for acrylamide biodegradation by several environmental consortia with different TEAs. These data were collected from the initial enrichment cultures before any serial dilutions were made. With TT as the source of microorganisms, the half-life of acrylamide under aerobic conditions is shorter (2.6 d) than under either sulfate-reducing (17 d) or methanogenic (116 d) conditions.

Similarly, with MFT, the half-life was shorter under sulfate-reducing conditions (9.9 d) than under methanogenic conditions (19 d). From Table 3.5, it can be seen that the half-lives in MFT-inoculated cultures were less than those in cultures inoculated with TT under both sulfate-reducing and methanogenic conditions. Although both inocula originate from the oil sands tailings, MFT have been settling in deposits for years, allowing for development of a more established microbial community. Conversely, TT have been only recently deposited, and so the numbers of microorganisms may not be as high as in MFT. Aging of TT likely will lead to an increase in microbial numbers, and an increased rate of acrylamide degradation.

Table 3.5. Summary of first-order kinetic data for acrylamide biodegradation in the presence of TEAs (oxygen, sulfate, carbon dioxide).

Incubation conditions	Inoculum	Initial acrylamide concn. ($mg\ L^{-1}$)	Lag time (d)	k_1 (d^{-1})	Half-life (d)	R^2
Aerobic[a]	TT	1.0	3	0.27	2.6	0.96
Sulfate-reducing	MFT	4.9	~0	0.07	9.9	0.88
	TT	6.2	~0	0.04	17	0.95
	Mud	5.6	<7	0.17	3.9	0.93
Methanogenic	MFT	5.2	<10	0.04	19	0.98
	TT	4.9	60	0.01	116	0.91

[a] in 0.8% saline medium

When acrylamide (1.2 mg L^{-1}) was added directly to TT under simulated field conditions, the half-life value under aerobic conditions was 0.5 d (Table 3.2), which was less than the value of 2.6 d observed in laboratory medium (Table 3.5). Under anaerobic conditions, the mean half-life under simulated field conditions was 2.7 d (Table 3.2). Both anaerobic conditions tested in laboratory medium, sulfate-reducing and methanogenic, caused longer half-lives of 17 d and 116 d, respectively. Thus, conditions in TT deposits may not be accurately represented by laboratory conditions, but the laboratory values give an indication of the persistence of acrylamide under defined conditions.

From these data, it is apparent that acrylamide at the low concentration predicted to occur in TT deposits (less than 20 μg L^{-1}) will not persist if the conditions remain aerobic for several days. Even after conditions become sulfate-reducing, acrylamide is removed in several weeks (Table 3.5). It is unlikely that acrylamide would remain until conditions in the TT deposit become methanogenic, but if this is the case, acrylamide may persist for a longer period. However, as stated previously, the microbial community in MFT is able to readily remove acrylamide, and so the long half-life demonstrated by TT may result from low numbers of methanogens.

3.2. PAM as nitrogen source for microorganisms

MFT (densified fine tailings derived from extraction tailings) and composite tailings (gypsum-treated extraction tailings) contain microbial communities that are capable of many different metabolic activities. Holowenko et al. (2001) and Fedorak et al. (2002, 2003) have demonstrated that the addition of various substrates can stimulate microbial activities. Similarly, the results reported in Section 3.1 have shown that microorganisms in TT and MFT are capable of degrading acrylamide.

Microorganisms require nitrogen for growth. PAM contains nitrogen and may serve as a nitrogen source for microbes (Kay-Shoemake et al. 1998a, b). If this is true, then the addition of PAM will stimulate microbial growth and activity. Although no amidases capable of acrylamide deamination have been characterized from anaerobic sources, numerous amidases produced by aerobic microorganisms have been purified and

characterized (see Table 1.3). If aerobic microorganisms deaminate acrylamide before oxygen becomes limiting, acrylic acid would be produced, which is mineralized to CO_2 and H_2O under aerobic conditions (Figure 1.5). The work described in the following sections explored the stimulation of microbial consortia supplemented with PAM as a nitrogen source.

3.2.1. PAM as a nitrogen source under aerobic conditions

Aerobic microorganisms capable of growth on PAM as a nitrogen source have been isolated by other groups, including *Enterobacter agglomerans*, *Azomonas macrocytogenes* (Nakamiya and Kinoshita 1995), *Pseudomonas* sp. (Grula et al. 1994), as well as a mixed culture enriched from agricultural soil previously treated with PAM (Kay-Shoemake et al. 1998a). Thus, it was anticipated that aerobic microbial consortia originating from oil sands may be able to use PAM as the sole nitrogen source.

Enrichment cultures were established to determine if microbial communities enriched from garden soil and Syncrude process water demonstrated the ability to use PAM (at a concentrations of 140 and 70 mg PAM L^{-1} for glucose- and acetate-containing cultures respectively) as a sole source of nitrogen (Section 2.2.3.1). Both sources of microorganisms demonstrated an ability to grow and consume acetate (Figure 3.18) or glucose (Figure 3.19) in the presence of PAM as a sole nitrogen source. When acetate was provided as a carbon source (1000 mg L^{-1}), cultures inoculated with microorganisms originating from garden soil showed a lag phase of about 14 d before acetate consumption began. Following this lag phase, acetate loss occurred rapidly, with a concentration of 100 mg L^{-1} reached by d 35. Microorganisms originating from process water demonstrated a lag phase of less than 12 d, with acetate consumption observed by d 12 (the first sampling time), and reached a minimum acetate concentration of 52 mg L^{-1} by d 43. Growth results correspond to acetate loss, although the OD_{600} readings were quite low in process water-inoculated culture, with a maximum of 0.17. However, cell clumping was observed in these cultures, which makes the OD results less representative of cell growth. Also, PAM is known to flocculate negatively-charged cells and other solids (Letey 2000), and so these measurements of cell growth are not accurate. Cultures inoculated with garden soil demonstrated a higher maximum OD_{600} of 0.64 at 43 d. Both

inocula demonstrated a low growth rate until d 14, which corresponds to the lag phase in acetate consumption.

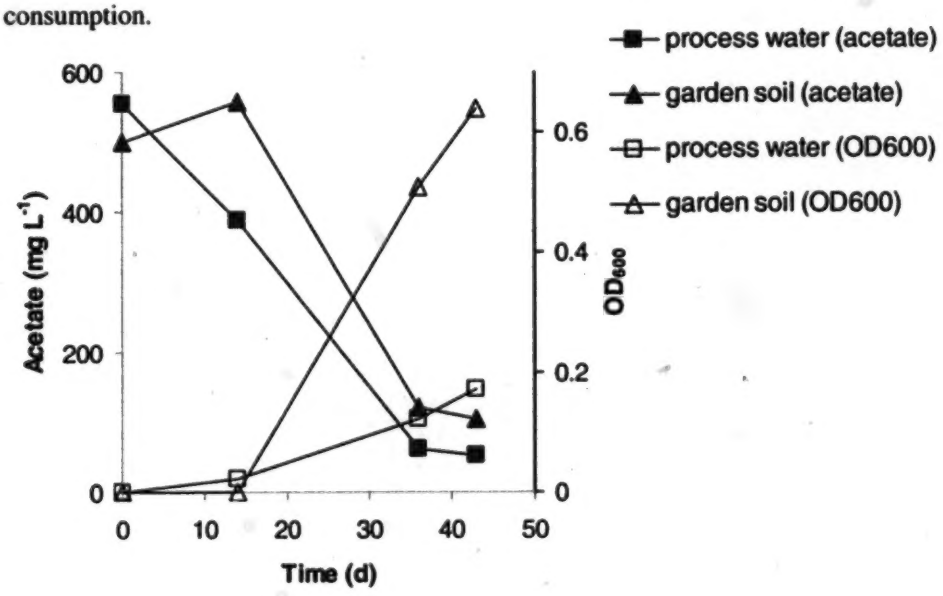

Figure 3.18. Growth and activity of enrichment cultures using PAM as sole nitrogen source and acetate as carbon source (after serial transfers of the original enrichment cultures).

When glucose was provided as a carbon source (1000 mg L^{-1}) (Figure 3.19), a lag phase of less than 14 d was observed for each source of inoculum. By d 14, 78% of the glucose had been removed from the culture containing microorganisms from garden soil, with a corresponding increase in the OD_{600} to 0.29. However, cultures inoculated with transfers of process water removed only 45% of the added glucose by d 14, and only 66% by d 36. Interestingly, the OD_{600} value increased only marginally to a maximum of 0.048 by d 43. No ammonium was detected in the medium in any of the cultures. However, this result is not unexpected, because the cultures were nitrogen-limited, with an initial substrate C:PAM-N ratio of 20:1. Thus, nitrogen released from PAM as ammonium would be rapidly used by cells as a nitrogen source. This nitrogen limitation may explain the concentration of 200 mg glucose L^{-1} remaining in the cultures after 43 d (Figure 3.19).

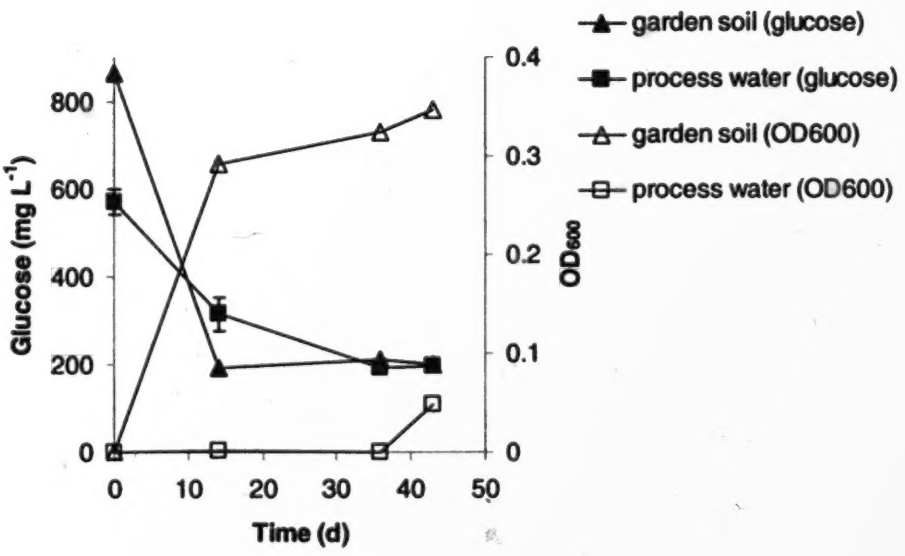

Figure 3.19. Growth and activity of enrichment cultures using PAM as sole nitrogen source and glucose as carbon source (after serial transfers of the original enrichment culture).

To verify that the nitrogen provided by PAM was responsible for the observed microbial growth, three techniques were employed: (1) a nitrogenase activity assay to rule out use of atmospheric dinitrogen, (2) acid hydrolysis of PAM nitrogen to see if there was a decrease in the amount of nitrogen bound to PAM (see Section 6.1.2), and (3) amidase assays to detect amidase activity in culture supernatants.

Although microbial growth was evident in cultures containing PAM as the only source of nitrogen, nitrogen fixation could account for such growth. A nitrogenase activity assay was attempted on cultures that demonstrated visible growth using the method described in Section 2.1.13, but the results of this assay provided little useful information, because the positive control failed to demonstrate nitrogen fixation (data not shown).

Acid hydrolysis appeared promising as a method to detect remaining nitrogen attached to PAM, as discussed in the Appendix 6.1.2. If PAM-N was being removed and used by microorganisms, the amount of nitrogen attached to the polymer was expected to decrease with time of incubation. However, this was not observed, because acid hydrolysis of samples taken from cultures revealed no detectable loss of nitrogen (data not shown).

Despite these inconclusive results, one final experiment provided indirect evidence that deamination of PAM is possible. An indirect method of detecting the possibility of PAM deamination is through amidase activity assays. The enrichment cultures used in this experiment had been given only PAM as a nitrogen source for approximately 2.5 y, so presumably the microbial consortia would be expected to produce extracellular amidases responsible for liberating the amide group from PAM, thereby providing ammonium for growth. If inducible amidases are produced by microorganisms in the presence of PAM, it was expected that amidase activity would be higher in cultures grown on PAM compared to the activity in cultures with no PAM, but with ammonium nitrate added as a nitrogen source. Thus, both ammonium release and extracellular protein production are expected to be higher in cultures grown on PAM. Figure 3.20 shows the results of an amidase assay designed to demonstrate the presence of extracellular amidases in these aerobic cultures.

More ammonium was released from two test amides (acetamide and acrylamide) when these were incubated with supernatant from cultures grown on PAM as the nitrogen source than from the supernatant of cultures that received ammonium nitrate (Figure 3.19). The highest concentration of ammonium was detected when acetamide was the test amide, with a concentration of 600 μM, which is 22 times higher than the concentration found in reaction mixtures incubated with supernatant from ammonium nitrate-containing cultures. The assay results from the test amide acrylamide also demonstrated an increased concentration of ammonium, although this concentration was lower than that shown in acetamide-containing reaction mixtures. Nevertheless, when acrylamide was the test amide, the concentration of ammonium detected was 3 times higher in reaction mixtures supplied with PAM-containing culture supernatant than in reaction mixtures containing ammonium nitrate-culture supernatant. No ammonium was

detected in the reaction mixture that contained PAM as the test amide and PAM-containing culture supernatant as the source of amidase.

Low concentrations of ammonium were detected in the reaction mixtures to which supernatant from ammonium nitrate had been added; in fact, all the reaction mixtures containing ammonium nitrate supernatant demonstrated similar concentrations of ammonium, with an average of 25 ± 2.1 μM. This was from carryover ammonium from the ammonium nitrate that was added as the culture nitrogen source, and likely not ammonium produced through amidase activity.

When extracellular protein production was measured, it was found that extracellular protein production was highest in cultures to which PAM had been supplied as a sole nitrogen source during growth (Figure 3.21). Cultures that had either ammonium nitrate or no nitrogen added demonstrated little extracellular protein production, because there was no induction by an amide-containing chemical such as PAM. Commercial amidase concentrations were determined to be ~ 25 μg mL^{-1} in reaction mixtures containing each test amide (data not shown).

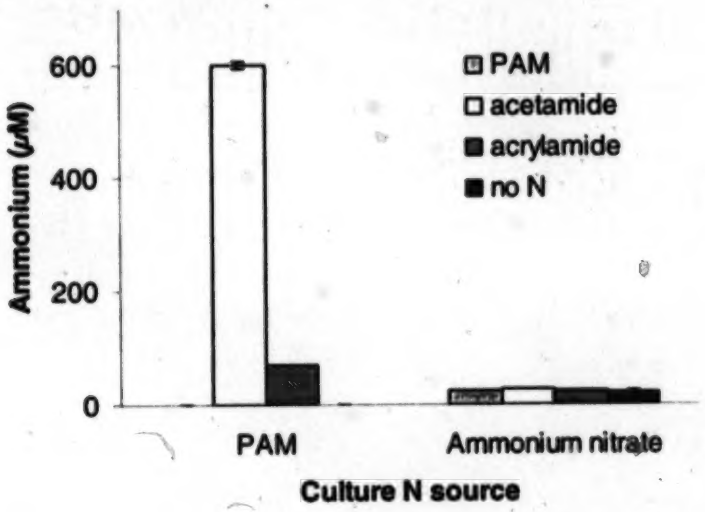

Figure 3.20. Ammonium released from presumed amidase activity produced by microorganisms enriched from oil sand process water after 7.5 h incubation. X-axis indicates the nitrogen sources in the cultures; test amides are shown in the legend.

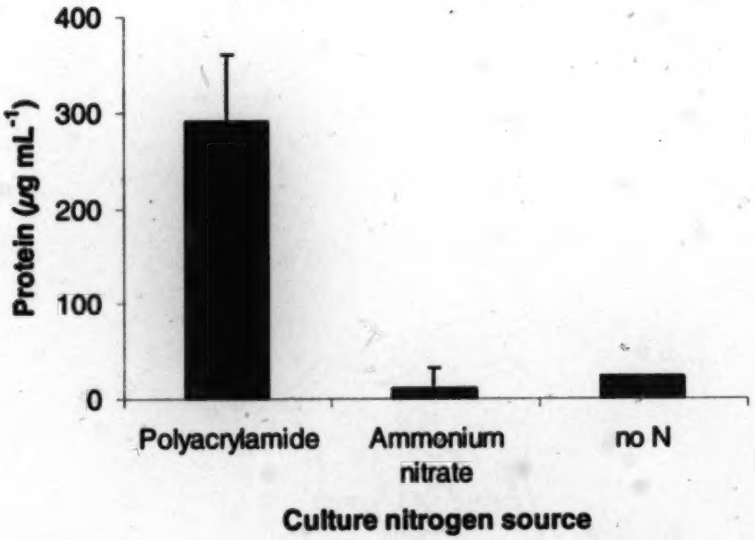

Figure 3.21. Extracellular protein content in acetamide-containing reaction mixtures containing supernatant from aerobic cultures grown on glucose with different nitrogen sources.

To ensure that the amidase assay was performing as desired, a parallel assay was performed using a commercial amidase. The results, shown in Figure 3.22, demonstrated that commercial amidase cleaved ammonium from acrylamide and acetamide, but not from PAM at a level significantly different from the control containing no added amide. However, background ammonium in the commercial amidase may have interfered with detection of small amounts of released ammonium from PAM. The absence of protein in reaction mixtures originating from ammonium nitrate-containing cultures verifies that the test amides are not being detected as protein.

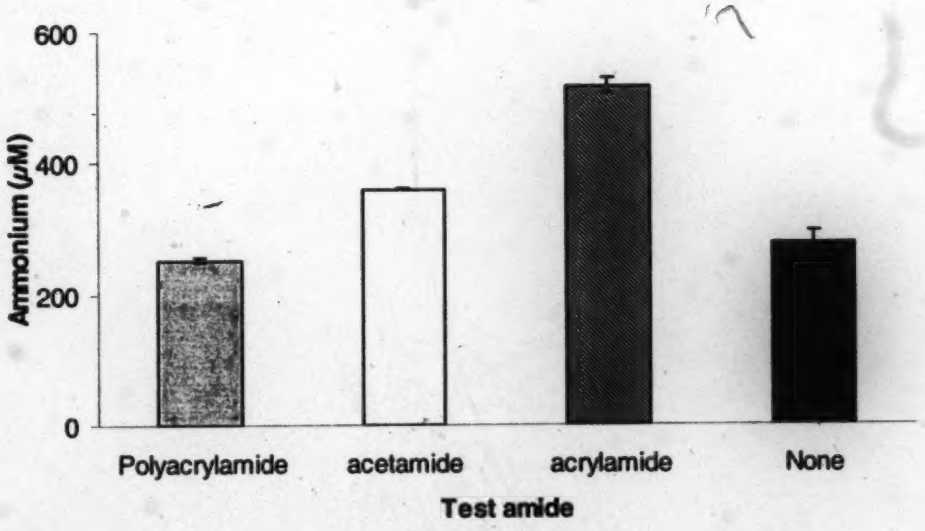

Figure 3.22. Ammonium release by a commercial amidase using different test amides.

No amidase activity was detected in reaction mixtures containing PAM as the test amide (Figure 3.22), which was surprising given the hypothesis that extracellular amidases are responsible for the ability of these cultures to grow when provided with PAM as sole nitrogen source. This is contrary to results reported by Kay-Shoemake et al. (1998a,b), who found that an extracellular amidase produced by a soil microbial consortium grown with PAM as a sole nitrogen source demonstrated amidase activity against PAM. However, this activity against PAM was lower than the activity observed against simple amides such as formamide and propionamide. Although these researchers stated that the PAM formulation used was free of contaminant nitrogen, no description of what methods were used to remove nitrogen, nor which forms of nitrogen were tested to verify this statement. Acrylamide is present at trace levels in PAM, and so if specially-treated PAM (washed to remove unreacted acrylamide) was not used in their amidase studies, the residual acrylamide present (up to 1000 mg acrylamide kg^{-1} PAM) may induce amidase production. Results reported in my study were obtained using methanol-washed PAM to reduce the acrylamide to concentrations less than 10 $\mu g\ L^{-1}$ (Semple et

al. 2001, R. Schaeffer, Ciba® Specialty Chemicals, personal communication, 2002), so amidase production was due to PAM, and not acrylamide.

The reaction mixtures containing PAM (Figure 3.20) were considerably more viscous than the other test amides, which may cause reduced enzyme mobility. Based on the results of the commercial amidase experiment (Figure 3.22), this may be the case, because even the purified enzyme did not produce a significantly higher concentration of ammonium over that in the amide-free control. The supernatant from the PAM-containing culture was also more viscous than that from the ammonium nitrate-containing culture. Thus, reaction mixtures containing PAM as the test amide and supernatant from PAM-containing cultures had increased viscosity originating from both ingredients in the reaction mixture, instead of only PAM. If reaction mixture viscosity effected in amidase activity, the activity in reaction mixtures containing supernatant from PAM-containing cultures would be even lower than that of the commercial amidase. This was observed, with the commercial amidase producing a concentration of 250 μM ammonium (Figure 3.22) compared to no ammonium in the reaction mixture containing culture supernatant (Figure 3.20). This lower ammonium production was not due to lack of amidase, because amidase activity was observed against the other two test amides (Figure 3.20). Cultures previously exposed to PAM as a nitrogen source (ie. during the 2.5 y enrichment), when later given a readily-usable nitrogen source such as ammonium nitrate, did not demonstrate significant release of ammonium from any of the test amides used (Figure 3.20). This result points to induced production of an extracellular protein. This protein is likely an amidase, because amidases are produced only in the presence of amide-containing chemicals (Brown 1980), and the production of amidases is repressed in the presence of ammonium.

The low level of protein detected in cultures containing either no nitrogen or ammonium nitrate may result from carryover of PAM from the original maintenance culture. The amide moiety may have induced a low level of amidase production, which would account for the protein detected. In fact, the lower concentration of protein in ammonium nitrate-containing cultures (compared to the cultures containing no nitrogen) can be explained by this repression as well, because cultures containing no nitrogen

would lack repression, and so would have a higher level of amidase protein. Although all the cultures were centrifuged to remove cells, the cultures containing either ammonium nitrate or no nitrogen were filtered (0.22 μm) before addition to the reaction mixtures. It is extremely unlikely that any extracellular proteins are larger than 0.22 μm, so the possibility of removal of any amidases that may have been produced is remote.

Numerous amidases from ubiquitous soil genera such as *Rhodococcus* spp. and *Pseudomonas* spp. have been characterized (Nawaz et al. 1993, 1994; Kumar and Kumar 1998), and amidase activity against PAM has been reported from mixed aerobic cultures originating from soil (Kay-Shoemake et al. 1998a,b). Further work to purify and characterize this enzyme would verify the identity of the protein as an amidase.

Although substrate removal and growth were evident in cultures containing PAM as the sole nitrogen source (Figure 3.18, Figure 3.19), experiments designed to demonstrate nitrogen originating from PAM was serving as the nitrogen source were inconclusive (data not shown). Kay-Shoemake et al. (1998b) also used the acid hydrolysis method of detecting PAM nitrogen, and did report nitrogen loss from the polymer backbone during a time course experiment. However, this group did not quantify the amount of nitrogen hydrolyzed, but instead reported the amount removed as a percentage of the time zero value. This may be problematic, because the method itself seems subject to variability (data not shown). Attempts were made to verify that dinitrogen fixation was not the source of nitrogen in these cultures, but these were inconclusive (data not shown).

The intent of these experiments under aerobic conditions was to develop methods for amidase detection that could be applied to anaerobic work. However, because of the limited success of this work, the methods were not applied to anaerobic cultures.

3.2.2. PAM as a nitrogen source under sulfate-reducing conditions

PAM is believed to serve as a nitrogen source for microorganisms (Kay-Shoemake et al. 1998a, b; Grula et al. 1994). Oxygen depletion in oil sands tailings will likely occur relatively rapidly compared to the slow usage of PAM nitrogen in the presence of more accessible nitrogen sources such as ammonium. Thus, PAM nitrogen will be available for use by anaerobic microorganisms, specifically SRB and

methanogens. To enrich for SRB, lactate was added as a substrate, because it is known to be used by many SRB, while not directly used by methanogens.

SRB originating from oil sands tailings consumed lactate in the presence of PAM, as observed in Figure 3.23a. However, there was no significant difference between lactate removal in cultures containing or lacking PAM. Cultures under both conditions demonstrated lactate removal following a lag phase of approximately 22 d, after which time rapid lactate removal commenced. By d 36, 79% and 88% of the lactate had been removed from cultures with no added nitrogen and with PAM added as the sole nitrogen source, respectively (Figure 3.23a).

Lactate metabolism by SRB yields acetate (Hansen 1993). Significant acetate production was observed in cultures containing PAM, as well as those lacking an added nitrogen source (Figure 3.23a). Similar trends were observed under both conditions, with no acetate detected at d 0, a maximum concentration near d 50, and gradual removal of acetate until a concentration of 0 was reached by d 109. Cultures containing PAM demonstrated significantly higher acetate concentrations throughout the experiment.

Sulfate loss was also detected in these cultures, as was expected if SRB were active and responsible for lactate removal (Figure 3.23b). Sulfate removal began within 15 d post-inoculation, and progressed rapidly. By d 36, 93% of the added sulfate had been consumed in cultures with no PAM, while 95% removal was observed in cultures with PAM. No sulfate loss was observed in killed controls, or in uninoculated controls. An unexplained increase in sulfate concentration was observed in the uninoculated controls.

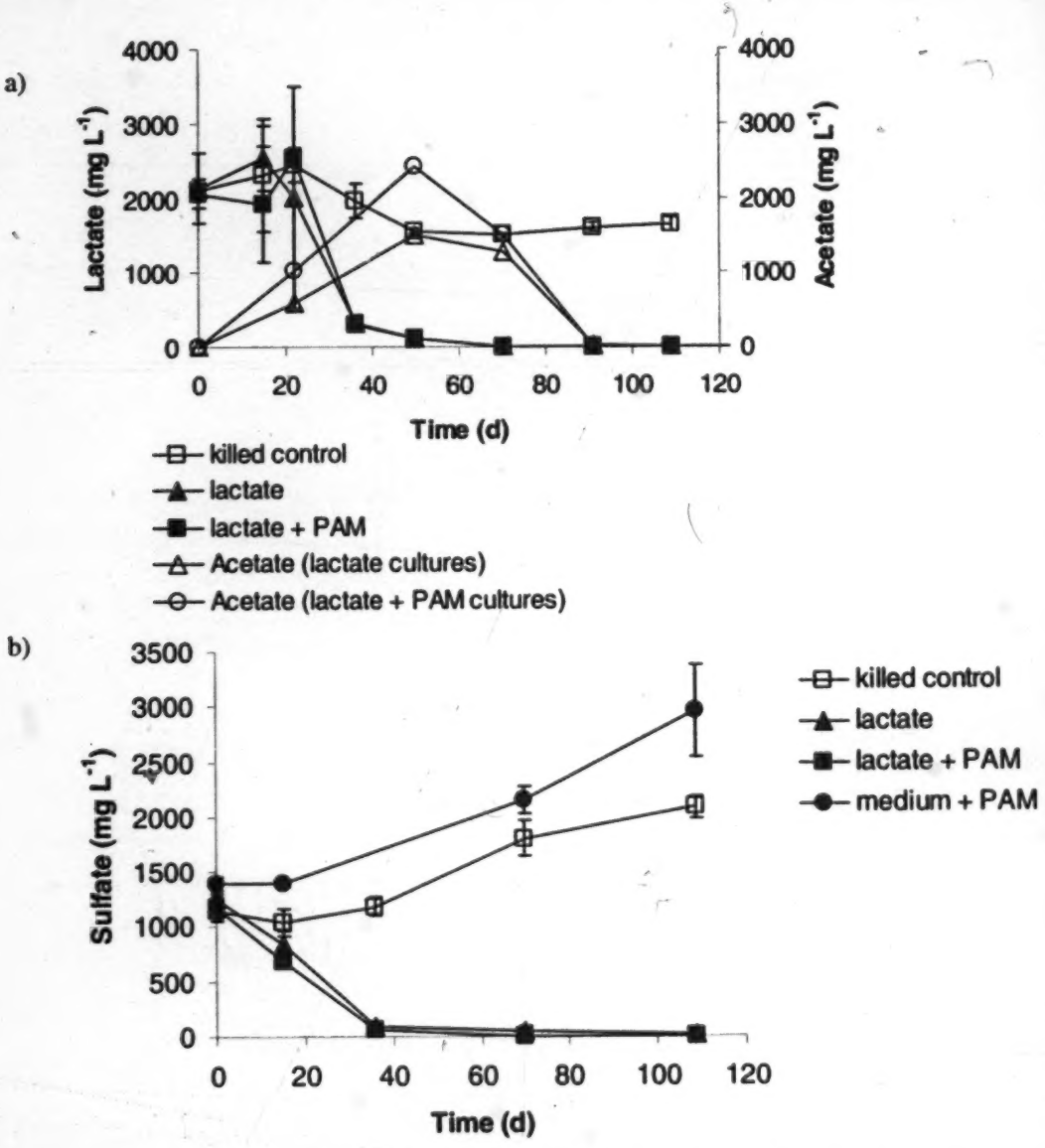

Figure 3.23. Activity of MFT-inoculated cultures incubated under sulfate-reducing conditions (original enrichment) a) lactate consumption and acetate production, b) sulfate removal.

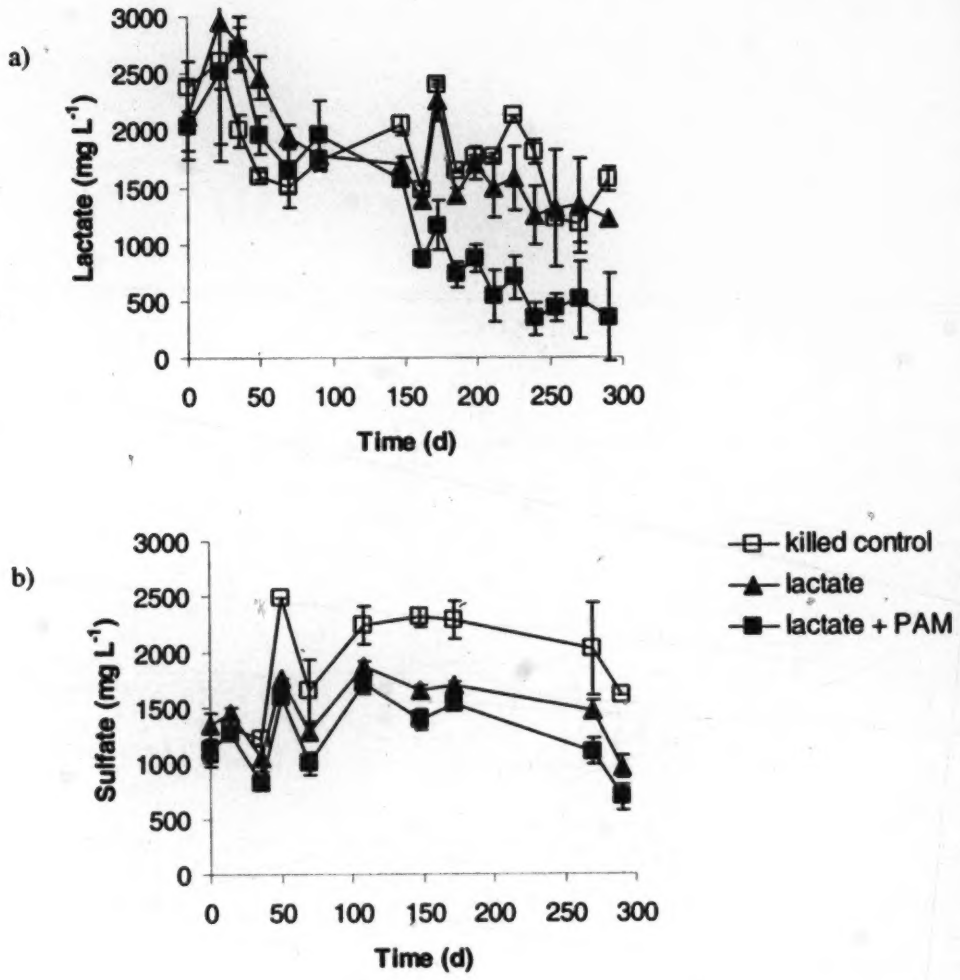

Figure 3.24. Activity of TT-inoculated cultures incubated under sulfate-reducing conditions (original enrichment) a) lactate consumption and b) sulfate removal.

Lactate consumption in TT-inoculated cultures demonstrated a lengthy lag period in comparison, as shown in Figure 3.24a. Whereas MFT-inoculated cultures removed lactate after 22 d (Figure 3.23a), TT-inoculated cultures demonstrated little

lactate loss prior to d 148 (Figure 3.24a). Furthermore, following d 148, TT-inoculated cultures supplemented with PAM demonstrated significantly more lactate removal than cultures with no added nitrogen source. A steady decline in the lactate concentration was observed, with a final concentration of 350 mg L^{-1} reached at the conclusion of the experiment (d 290). This value was considerably lower than those in cultures lacking PAM and the killed controls, which had mean concentrations of 1220 mg L^{-1} and 1570 mg L^{-1} respectively. This is in direct contrast to MFT-inoculated cultures, in which lactate was removed both in cultures containing or lacking PAM. No acetate analyses were performed on TT-containing cultures.

However, despite lactate loss in PAM-supplemented cultures, little sulfate loss was observed in these cultures (Figure 3.23b). By the end of the experiment, only 39% of the added sulfate was consumed in PAM-containing cultures, which was similar to the 28% use in PAM-lacking cultures. If complete mineralization of lactate had occurred, a loss of 1.6 mmol lactate (as was seen in PAM-containing cultures) would require 2.4 mmol of sulfate if lactate was consumed solely by SRB, according to Equation 3.1. The actual sulfate loss was 0.43 mmol, which is 18% of the expected value. Even if SRB were present which cannot completely oxidize lactate (see Equation 3.2), the amount of sulfate required would be 0.81 mmoles, which is only approximately half of that required for the amount of lactate consumed.

$$CH_3CHOHCOO^- + 1.5SO_4^{2-} + 4H^+ \rightarrow 3CO_2 + 1.5H_2S + 3H_2O$$

Equation 3.1. Equation for complete mineralization of lactate by SRB

$$2CH_3CHOHCOO^- + SO_4^{2-} \rightarrow H_2S + 2CH_3COO^- + 2HCO_3^-$$

Equation 3.2. Equation for production of acetate from lactate by SRB.

Therefore, theoretically it is possible that sulfate reduction occurred, but it appears that some other mechanism was responsible for lactate usage in these cultures. One possibility is that propionate-forming bacteria may be active in these cultures, as such microorganisms have been detected in oil sand tailings (Holowenko 2000). These

microorganisms form propionate from lactate, with no associated sulfate loss. No propionate analysis was performed to verify this hypothesis. However, no matter which microorganisms were responsible for the lactate consumption, it appears that something in the PAM-containing cultures enhanced lactate use. This could be related to the observations of Grula et al. (1994), who observed growth stimulation of mixed cultures of SRB originating from oil field floodwaters in the presence of PAM. These researchers also discovered that SRB MPN values were significantly higher in an oil field previously exposed to PAM than in non-flooded wells (Grula et al. 1994). In addition, PAM allowed limited growth of SRB, but only 0.1% of a ^{14}C-labeled PAM provided as a sole carbon source was cell-associated. Thus, Grula et al. (1994) concluded that PAM likely served as an electron source for sulfate reduction, but did not serve directly as a carbon source.

During sampling in latter days of the experiment, considerable headspace pressure was observed in microcosms inoculated with either MFT or TT. Methane analyses were performed to determine if methanogenesis was responsible for the headspace pressure, and the results are shown in Table 3.6. Significantly higher methane production was observed in cultures supplemented with PAM in MFT- or TT-inoculated cultures. TT-inoculated cultures demonstrated the most striking difference between cultures containing PAM and cultures lacking PAM, with a mean difference of 16.9% (vol.) methane between the two conditions.

Table 3.6. Methane observed in original enrichments of "sulfate-reducing" cultures with PAM as sole nitrogen source and lactate as carbon source.

	Methane (% vol.)	
	MFT (d 191)	TT (d 186)
Killed control	0.08 ± 0.06[a]	0
No PAM	13.9 ± 0.7	8.6 ± 2.5
PAM	18.2 ± 1.2	25.5 ± 1.5

[a] indicates standard deviation of n=3.

Once oxygen was depleted from the TT deposit, sulfate-reducing conditions will predominate, and so experiments were undertaken to investigate the ability of environmental microorganisms to utilize PAM as a nitrogen source with lactate as a carbon source. Although lactate was consumed in cultures inoculated with MFT, there was no significant difference in sulfate loss between cultures containing PAM as a nitrogen source and those lacking any added nitrogen source, because both demonstrated complete sulfate loss in 36 d (Figure 3.23). Thus, PAM use as a nitrogen source could not be coupled to sulfate loss or lactate consumption. Subsequent methane production was observed, indicating that the cultures were no longer under sulfate-reducing conditions.

Acetate is the most commonly-used substrate for methanogens (Ferry 1993); however, many SRB also use acetate as a carbon source (Hansen 1993). Considering thermodynamics, sulfate reduction provides more energy per mol of acetate, as can be seen from the more negative $\Delta G^{o'}$ value for SRB shown in Table 3.7. Given this favorable bioenergetic yield from acetate by SRB, it is expected that acetate will be used by SRB rather than methanogens. In addition, SRB have a higher substrate affinity for acetate than do methanogens, allowing SRB to outcompete methanogens under conditions where substrate is limited (Schönheit et al. 1982).

Table 3.7. Free energy available from acetate utilization by SRB and methanogens (adapted from Zinder 1993)

Process	Equation	$\Delta G^{o'}$ (kJ)[a]
Sulfate reduction	$CH_3COO^- + SO_4^{2-} \rightarrow HS^- + 2HCO_3^-$	- 47
Methanogenesis	$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^-$	- 31

[a] values from Thauer et al. (1977)

However, once sulfate has been depleted in the surrounding environment, methanogenesis will become the predominant metabolic activity. Methane analysis was not conducted on MFT-inoculated cultures throughout the experiment, but methanogenesis was evident at the end of the experiment (Table 3.6). If all of the acetate

detected at the maximum detected concentration (d 50) was converted to methane, a maximum of 60% (vol.) methane was expected, according to Buswell's equation (Equation 2.1). However, as of the last sampling date (191 d), about one-third of the expected methane had been produced. Methane formation in the presence of sulfate has been reported by several groups (Mitterer et al. 2001, Mizuno et al. 1994, Oude Elferink et al. 1994), so there is no requirement for complete sulfate depletion prior to methane formation.

In order for SRB to use PAM as a nitrogen source, extracellular amidases must be present in the environment to cleave the amide group from the polymer. Although the literature contains no reports specifically measuring amidase activity in SRB, Boopathy and Kulpa (1992) isolated a *Desulfovibrio* sp. capable of reducing the nitro groups of trinitrotoluene (TNT), 2,4-dinitrophenol, 2,4-dinitrotoluene, and 2,6-dinitrotoluene to amines. Then this organism deaminated the amino groups to release ammonium (Boopathy and Kulpa 1992, Boopathy and Kulpa 1993). Although amidase assays were not performed, it appeared that amidases may be responsible for this activity.

Although *D. desulfuricans* B was reported to use certain nitroaromatics as sole source of nitrogen, later studies found that a defined consortium of four *Desulfovibrio* spp. was required to reductively deaminate related compounds (Boopathy et al. 1998). When tested in isolation, none of the four species (*D. desulfuricans* A, *D. desulfuricans* B, *D. gigas*, and a strain closely resembling *D. vulgaris*) was effective. When tested together, the consortium was able to reductively deaminate three nitroaromatic explosive compounds related to TNT with resulting ammonium release (Boopathy et al. 1998).

Although individual SRB may be capable of producing amidases, amidase production may not occur in the absence of an unknown growth factor, perhaps provided by another member of a consortium. However, the work of Boopathy and Kulpa (1992, 1993) and Boopathy et al. (1998) demonstrate that some SRB are capable of deamination. Of course microorganisms other than SRB may have produced amidases in my consortia, and the released ammonium could have been consumed by the SRB.

3.2.2.1. PAM as a nitrogen source under methanogenic conditions

The data in Table 3.6 showed that methane was detected in MFT and TT cultures after approximately 190 d incubation. These results also show that more methane was produced in the presence of PAM, indicating that PAM may serve as a nitrogen source in methanogenic consortia. Thus, three carbon sources (acetate, benzoate, and glucose at 1000 mg L^{-1}) were chosen to test the ability of a wide variety of methanogenic consortia to use PAM as a nitrogen source.

Most biogenic CH_4 is produced from acetate, using one of two known pathways. Methanogens can either reduce the methyl group of acetate to produce methane, or can reduce CO_2 with electrons originating from H_2 or formate (Ferry 1993). Thus, acetate provided a carbon source that a wide variety of methanogens could use. Benzoate is also a commonly-used carbon source for methanogenic consortia (Londry and Fedorak 1992). Although glucose was also tested, the amount of methane produced was approximately a third of the expected theoretical amount of 30% (vol.) (according to Buswell's equation). The reason for this is unknown, and the results from this experiment are given in Appendix A, Section 6.2).

When enrichment cultures were established with the three environmental consortia (MFT, TT, and sewage sludge), some nitrogen originating from the inocula was likely present. Enhanced methanogenesis resulting from PAM-N in most of these original enrichment cultures was not detected, likely because of residual nitrogen from sources other than PAM that allowed growth. Cultures inoculated with sewage sludge demonstrated no enhanced methanogenesis in the presence of PAM with either acetate or benzoate, whereas MFT and TT cultures demonstrated similar methane production with acetate as a carbon source (data not shown). Once 10-mL volumes of these original enrichment cultures were serially transferred into 85 mL of fresh medium, the effect of this residual nitrogen was minimized, because a) readily-usable nitrogen, such as ammonium, was previously consumed, and b) any remaining nitrogen was diluted by factors of 1/8.5, 1/72, and 1/614 following first, second and third transfers, respectively. Because of these factors, only results of later transfers are presented to highlight differences between cultures containing PAM and those lacking added nitrogen.

However, two notable exceptions to this trend were observed in cultures inoculated with TT or MFT and provided with benzoate as a carbon source. Both benzoate loss and methane production were observed in viable MFT-inoculated cultures that contained PAM, with a maximum methane concentration of 39 ± 5% (vol.) reached prior to transfer into fresh medium (data not shown). However, 25 ± 4% (vol.) methane was observed in cultures with no added nitrogen (data not shown).

In contrast, in TT-inoculated viable cultures, only cultures containing both PAM and benzoate produced methane (data not shown). A lag phase approximately 91 d was observed for both methane production and benzoate loss, after which methane production increased to a maximum of 24 ± 8% (vol.) by d 161, which corresponded to benzoate consumption.

Although few significant differences were observed in early enrichment cultures, later transfers confirmed that enhanced methane production was observed in viable cultures containing PAM inoculated with each of the three environmental sources. Representative data that highlight recurring trends have been selected for presentation. MFT and TT demonstrated very similar trends in methane production and substrate utilization. TT originated from the Aurora site at Syncrude Canada Ltd., where an experimental thickener field test was run in 2001 (Matthews et al. 2002). Thus, where appropriate, TT results are shown.

Acetate provided a readily-usable carbon and energy source for methanogens present in both sewage sludge and TT, as shown in Figure 3.25 and Figure 3.26. Cultures derived from serial dilutions of sewage sludge or TT demonstrated significant acetate loss only in the presence of polyacrylamide (Figure 3.25a and Figure 3.26a). Sewage sludge-inoculated cultures demonstrated a lag period of about 14 d prior to onset of acetate removal, after which time acetate was removed at a rapid rate of 14 mg L^{-1} d^{-1} (Figure 3.25a). From d 70 to 83 there was a slight lag, then acetate removal resumed at a slower rate of 5 mg L^{-1} d^{-1} until complete removal at d 194.

No lag was observed in methane production, with methane increasing steadily to d 105, after which time the rate slowed slightly (Figure 3.25b). The maximum amount of methane detected was approximately ~30% (vol.) methane, which was reached on d 156. Following d 156, the methane production reached a plateau. Although a small amount of

methane was produced in cultures containing acetate but lacking PAM, this amount reached a maximum of only 6% (vol.) by the conclusion of the experiment (d 269), which is approximately 1/5 of the amount produced in cultures containing PAM. Thus, any residual nitrogen, which by this transfer has been diluted by 1/614, is having a negligible effect on methanogenesis. No methane was produced in killed controls containing PAM, so the methane produced is of biological origin. Viable acetate-free controls produced no methane over the course of the experiment. In particular, the absence of methane in the acetate-free viable control containing PAM is significant, as this suggests that this particular methanogenic consortium is unable to use PAM as a carbon source.

Cultures originating from serial dilutions of TT demonstrated similar results to those of sewage-inoculated cultures, as seen in Figure 3.26. However, TT-inoculated cultures containing PAM displayed a longer lag period of approximately 42 d prior to beginning acetate removal (Figure 3.26a). The large error bars reflect a disparity in one of the three replicates that displayed significantly slower removal than did the other two replicates. Once acetate degradation began, the rate remained relatively constant, with complete loss in two of the three replicates by d 170. No acetate loss was detected in killed controls, and the acetate concentration in PAM-free viable cultures containing added acetate was determined to be not significantly different from that of the killed control.

Methane production corresponding to acetate loss was observed in TT-inoculated cultures containing PAM, as seen in Figure 3.26b. A lag period of 28 d was observed, after which time methane production increased rapidly to a maximum of ~32% (vol.) methane reached by d 163. As was seen in sewage sludge-inoculated cultures (Figure 3.25), methane was produced in PAM-free viable cultures containing acetate, but this value was only 8% (vol.) by d 219, which was one-quarter of the value observed in cultures containing PAM. PAM did not serve as a source of carbon, because no methane was detected in viable acetate-free controls.

Benzoate was chosen as a carbon source to enrich for a methanogenic community able to use PAM as a sole nitrogen source, rather than testing only acetate-utilizing methanogens. Viable cultures inoculated with TT removed benzoate and produced

methane only in the presence of PAM and benzoate, as is seen Figure 3.27a and b. Benzoate removal began within 7 d post-inoculation, with nearly complete removal achieved by d 79. Similarly, methane production began nearly immediately, with a maximum value of 21 ± 2% (vol.) methane reached at the conclusion of the experiment. Little methane was produced in viable cultures lacking PAM, meaning that there was minimal residual nitrogen remaining from the inoculum. Nor was any significant methane production observed in killed controls or viable benzoate-free controls. The latter result indicates that PAM did not serve as a carbon source for this TT methanogenic consortium, which mirrored the results of the acetate-containing cultures (Figure 3.25 and Figure 3.26).

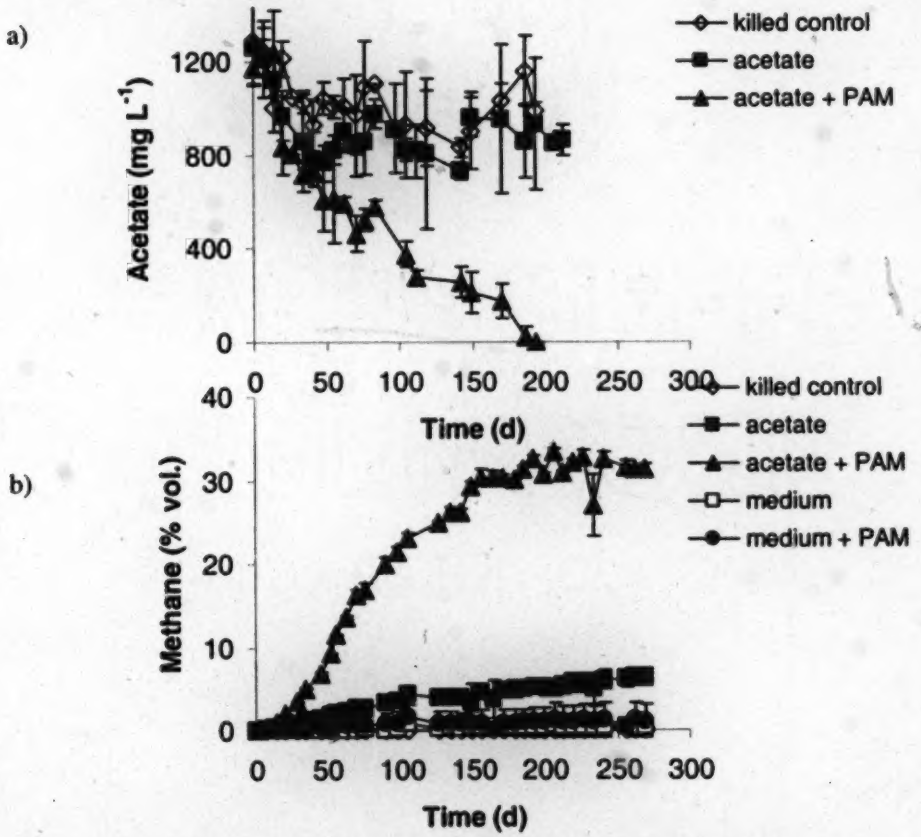

Figure 3.25. Activity of microcosms containing sewage sludge with acetate as carbon source and PAM as nitrogen source (transfer 3). a) acetate removal, b) methane production. Error bars represent standard deviation of n=3.

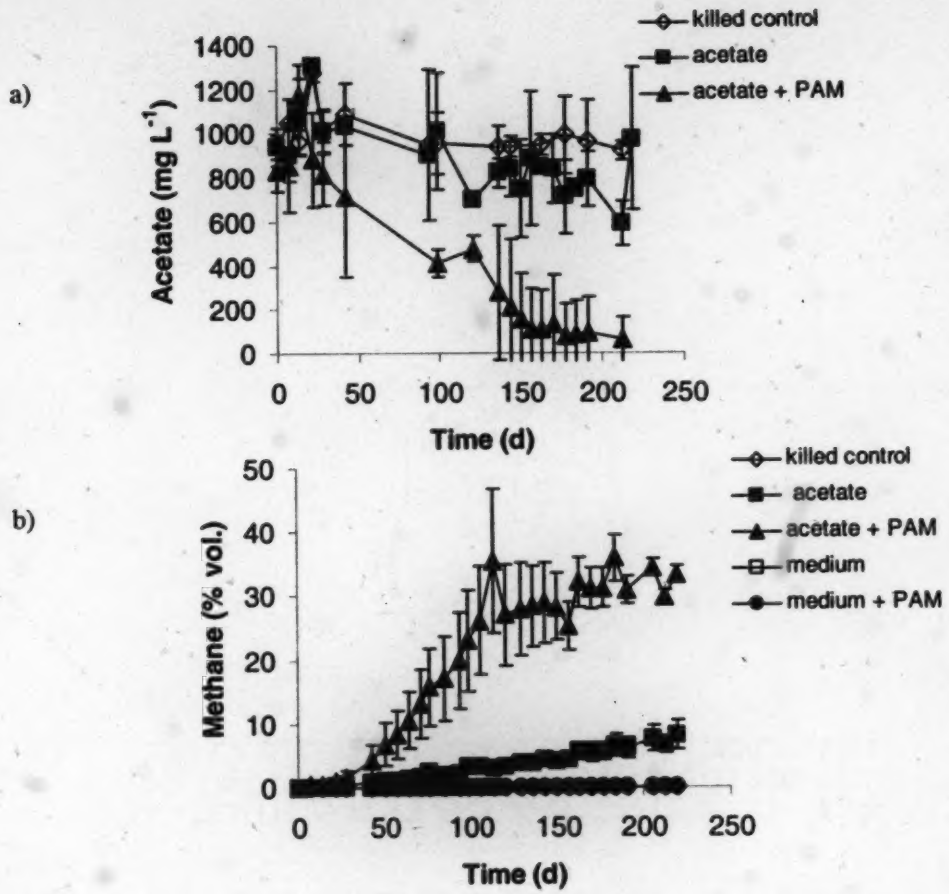

Figure 3.26. Activity of microcosms containing TT with acetate as carbon source and PAM as nitrogen source (transfer 3). a) acetate removal, b) methane production. Error bars represent standard deviation of n=3.

Ammonium was quantified in the PAM-containing viable cultures, but no significant increases were detected over the course of the experiment (data not shown), which is expected under the conditions of nitrogen limitation imposed on these cultures.

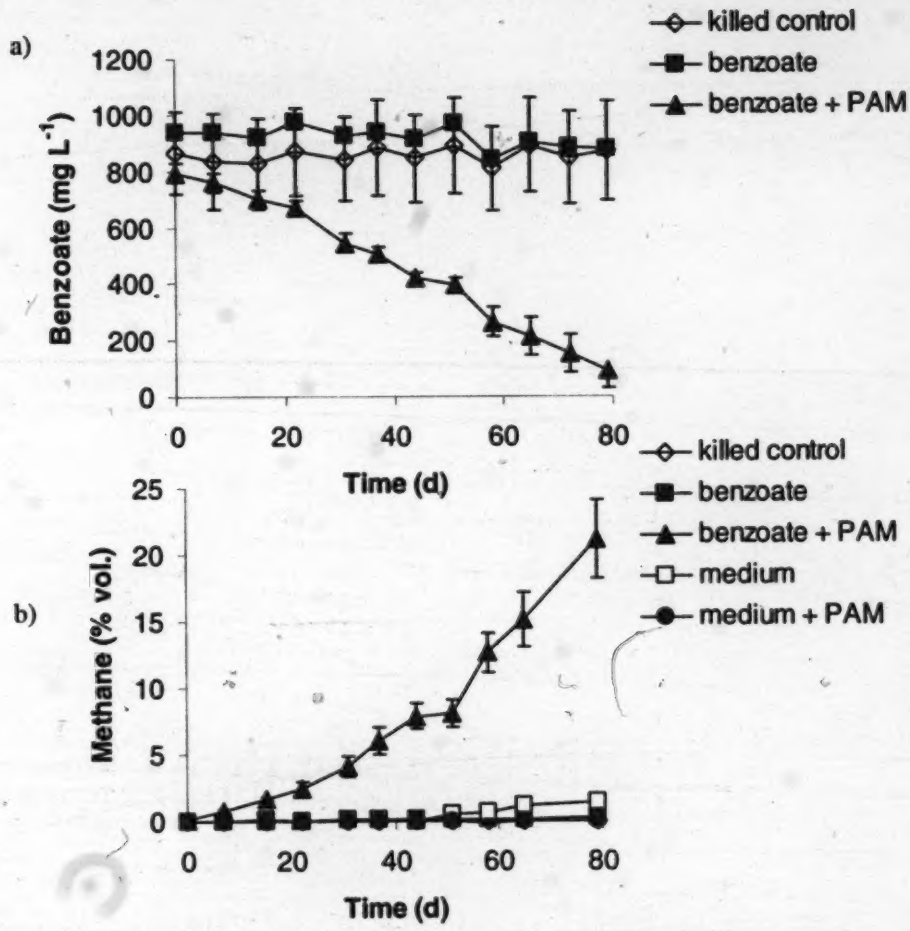

Figure 3.27. Activity of TT-containing microcosms with benzoate as carbon source and PAM as nitrogen source (transfer 1). a) benzoate removal, b) methane production. Error bars represent standard deviation of n=3.

Initial enrichments with sewage sludge as an inoculum demonstrated no significant differences in methane production between cultures containing PAM and those lacking any added nitrogen (data not shown). By the second transfer, significantly more methane was evident in cultures containing PAM than in killed controls, viable carbon-free controls, or viable cultures with no added nitrogen (Figure 3.28b). After a

lag time of approximately 22 d, methane production began, with a maximum value of 25% (vol.) reached at d 270.

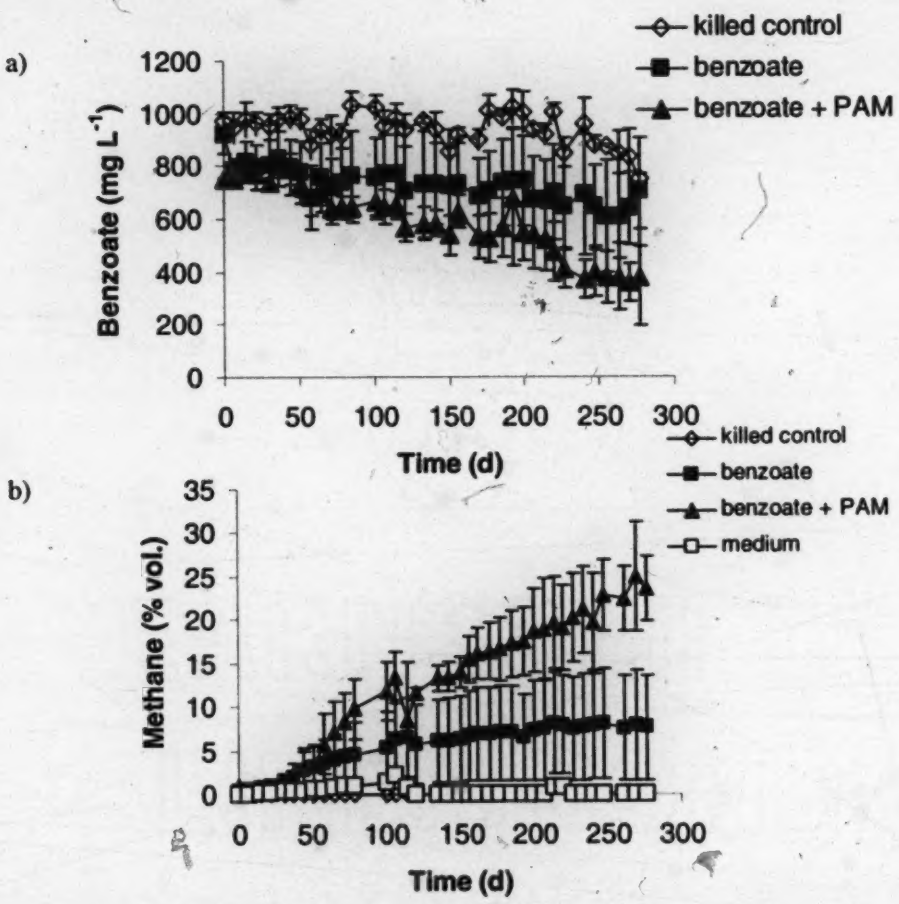

Figure 3.28. Activity of sewage sludge-containing microcosms with benzoate as carbon source and PAM as nitrogen source (transfer 2). a) benzoate removal, b) methane production. Error bars represent standard deviation of n=3.

There was some benzoate loss (370 mg L^{-1}) coupled to enhanced methane production in cultures containing both benzoate and PAM (Figure 3.28b), although not all the benzoate was consumed. No benzoate removal was observed in either killed controls or PAM-lacking viable cultures.

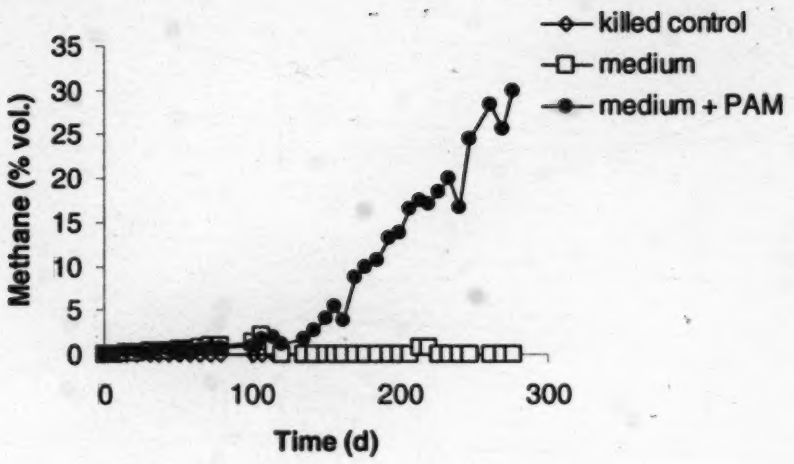

Figure 3.29. Methane production by a microcosm inoculated with sewage sludge and containing PAM as a sole source of carbon and nitrogen.

Surprisingly, methane production was observed in the sole benzoate-free viable control in this same experiment (Figure 3.29). After a lag period of about 121 d, this control rapidly produced methane at a rate and to an extent that surpassed all the other sewage sludge-containing microcosms (Figure 3.28b). A maximum value of 30% (vol.) was reached at d 277 (Figure 3.29), and which subsequently remained constant thereafter (data not shown). This viable control was transferred in the same manner as the other conditions, so carbon carryover could not account for the amount of methane produced.

To verify that PAM-C was metabolized, carbon and viscosity measurements were performed on samples of the benzoate-free viable control that demonstrated methane production, as well as on a killed control. Although the killed control had been autoclaved, which may have caused thermal degradation of the polymer, all the PAM-C added should be contained in the sealed serum bottle and therefore detected by total carbon analysis. The results of these analyses are given in Table 3.8. Because the killed control contained benzoate in addition to PAM, the amount of carbon provided by benzoate was calculated and subtracted from the analyzed organic carbon value. According to the results, the concentration of organic carbon was significantly lower in

the viable culture when compared to the killed control, indicating that loss of carbon has occurred.

Table 3.8. PAM-C use in microcosms inoculated with sewage sludge provided with different carbon sources after two serial transfers (d 390).

	Carbon source	Carbon ($mg\ L^{-1}$)			
		Total	Organic	Organic (corrected)	Viscosity ($cSt\ s^{-1}$) [c]
Killed control	Benzoate, PAM	1420±8[b]	850±11	340±11[a]	0.589±0.004
Viable culture	PAM	780±9	170±4	170±4	0.490±0.005

[a] calculated by subtracting 510 mg benzoate-C L^{-1} (calculated from benzoate concentration in killed control on last sampling date (d 277)) from organic-C

[b] represents standard deviation of n=3

[c] all samples were diluted 1/5; viscosity of water was 0.458±0.001 $cSt\ s^{-1}$ (see Section 2.1.11)

Viscosity has been used to measure PAM degradation in previous studies by other researchers (Grula et al. 1994, Grula et al. 1982), and so attempts were made to determine if viscosity was reduced in the viable culture when compared to the killed control. One potential confounding factor in this measurement was the autoclave treatment of the killed control, as thermal degradation may have occurred. However, despite this factor, the viscosity of the viable culture was significantly less than that of the killed control, indicating that some degree of polymer degradation has occurred. Following this discovery, all other acetate-, benzoate-, and glucose-free viable controls inoculated with sewage, MFT, or TT (~ 24 cultures) which contained PAM from previous enrichments were tested for methane production, and none produced significant amounts of methane. Thus, it appears that only this single culture demonstrated this activity.

To verify that PAM-N was serving as a nitrogen source, and that nitrogen fixation was not occurring, an experiment containing argon as the headspace gas was established. The methane concentration in cultures containing ammonium as a nitrogen source was

highest, at 14% (vol.) (Figure 3.30). If dinitrogen was the nitrogen source used by these microbes in these methanogenic microcosms, the concentration of methane in the argon-containing microcosm would be substantially lower than that in the nitrogen-containing microcosm. However, there was virtually no difference between the methane production in these two microcosms (Figure 3.30). Because the amount of inoculum for these microcosms was limited, no replicates were prepared and the results cannot be statistically compared.

Nitrogen fixation by methanogens has been well-documented, with nitrogen fixing activity reported in *Methanosarcina barkeri* (Bomar et al. 1985, Lobo and Zinder 1988, 1990), *Methanococcus thermolithotrophicus* (Belay et al. 1984, 1988), *Methanobacterium bryantii*, and *Methanospirillum hungatei* (Belay et al. 1988). However, detection of such activity is not simple, because acetylene reduction assays commonly used to demonstrate nitrogenase activity are often inhibitory to methanogens (Oremland and Taylor 1975, Sprott et al. 1982). However, if nitrogen fixation was responsible for the increased methanogenesis, viable cultures lacking any additional nitrogen would have stimulated methanogenesis to similar levels as PAM. This situation was not observed in any of the later transfers (Figure 3.25b, Figure 3.26b, Figure 3.27b, Figure 3.28b).

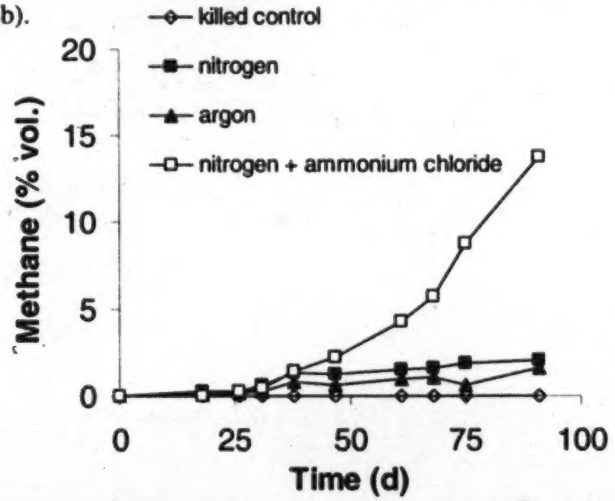

Figure 3.30. Methane production in microcosms inoculated with serially diluted sewage sludge supplied with acetate as a carbon source with various headspace gas compositions.

To provide direct evidence that PAM is serving as sole nitrogen source, attempts were made to quantify ammonium release from cultures inoculated with TT containing benzoate as carbon source. If PAM is deaminated through the pathway proposed for acrylamide (Shanker et al. 1990), ammonium should be detected in the culture medium. No ammonium was detected despite complete benzoate loss and methane. However, any ammonium produced was likely taken up rapidly by microbial cells, because nitrogen was limiting in these cultures.

From the data gathered in Section 3.2.2.1, it is apparent that PAM is likely serving as a sole source of nitrogen in methanogenic consortia. Table 3.9 summarizes the results of these experiments. Although early enrichments demonstrated no significant differences in methanogenesis between cultures containing or lacking PAM, the results of later transfers, in which nitrogen carryover from inocula was minimal, were more conclusive (Table 3.9). PAM, when added as the sole nitrogen source, stimulated methanogenesis with all inocula tested. When compared to cultures with no added nitrogen source, PAM-containing cultures produced more than twice as much methane in all cultures except the culture inoculated with sewage and provided with acetate as a carbon source. One striking result is the very high ratio of 92 observed in cultures inoculated with TT and supplied with benzoate (Table 3.9). Negligible amounts of methane were produced by the culture that contained no PAM in this transfer, and even in the original enrichment (data not shown), proving that carryover of other nitrogen sources was minimal.

The maximum amount of methane produced in acetate-containing cultures inoculated with MFT, TT, and sewage were very similar, with an average of ~30% (vol.), which corresponds closely to the theoretical value of 31% calculated using Buswell's equation (see Equation 2.1 and Table 2.2). Methanogenesis in benzoate-containing cultures was also enhanced upon addition of PAM, but maximum methane values reached approximately half of the calculated theoretical value of 46% (vol.).

Table 3.9. Enhanced methanogenesis by environmental consortia supplemented with PAM as sole nitrogen source over controls.[a]

Carbon source	MFT		TT		Sewage	
	Enhanced CH_4[b]	Maximum CH_4[c] (d reached)[d]	Enhanced CH_4	Maximum CH_4 (d reached)	Enhanced CH_4	Maximum CH_4 (d reached)
Acetate	2.7	30±2% (126)	2.2	29±2% (125)	1.2	31±2% (98)
Benzoate	2.7	19±2% (167)	92	21±3% (79)	2.9	13±4% (248)

[a]all data shown is from serial transfer 2, except MFT and TT benzoate (transfer 1).

[b] ratio of methane in PAM-containing cultures to methane in PAM-lacking cultures

[c] in % (vol.) ± standard deviation of n=3.

[d] days of incubation before maximum methane concentration was reached

Although PAM was not believed to be capable of serving as a carbon source for microbial communities under any conditions, the results of the single sewage-inoculated viable control containing PAM as the only source of added carbon raised this possibility (Figure 3.29). Typically, degradation of water-soluble synthetic polymers with carbon backbones such as PAM is not significant until the average molecular weight is reduced to below 1000 (Larson et al. 1997, Swift 1994). Biodegradation of intact high-molecular weight PAM has been demonstrated to be minimal, with mineralization of less than 1% (Grula and Huang 1981, Grula et al. 1994, Kay-Shoemake et al. 1998a).

The effect of cleaving high molecular weight PAM into smaller oligomers on biodegradation has produced conflicting reports in the literature. Chain length reduction by chemical or physical means was shown to increase biodegradation only marginally (Kay-Shoemake et al. 1998b; Soponkanaporn and Gehr 1989; Suzuki et al. 1978). However, El-Mamouni et al. (2002) subjected a nonionic ^{14}C-labeled PAM to UV irradiation, and found that 79% of the polymer was lyzed into fragments less than 1 kD

after 72 h of treatment. Such degradation was shown to allow production of 8 to 10% (vol.) methane, which is considerably less than the level of 30% observed in this study.

Under methanogenic conditions, evidence for nitrogen removal was provided in Section 3.2.2.1. Once PAM is completely deaminated, polyacrylic acid (polyacrylate) is produced. Several research groups have observed mineralization of polyacrylate after some degree of carbon backbone hydrolysis. Larson et al. (1997) reported that oligomers of 500 and 700 MW were degraded by an activated sludge consortium to produce 70 to 80% of the DOC detected as CO_2. An *Arthrobacter* sp. capable of degrading polyacrylate oligomers smaller than hexamers was isolated by Hayashi et al. (1993). The mechanism for such action has been proposed as β -oxidation (Hayashi et al. 1993, Kawai 1995).

Scission of the carbon backbone of PAM leads to some depolymerization (Gurkaynak et al. 1996). During transport of TT from the clarifier to their destination in deposition pits, some degree of shear will likely occur. Therefore, the extent of polymer degradation resulting from mechanical breakage must be investigated in this situation, and future experiments should address the possibility of biodegradation of fragments of suitable MW.

4. Conclusions and Implications

4.1. Acrylamide degradation

Acrylamide has historically been classified as a potential neurotoxic, genotoxic, and carcinogenic chemical (Dearfield et al. 1988). To minimize risk to public health, standards for acrylamide in PAM used for potable water treatment have been set at 0.05% (w/w) (US EPA 1994). However, recent findings have determined that acrylamide is formed in many foods as a result of cooking; in particular, plant-derived foods rich in asparagine contain high concentrations of acrylamide (Mottram et al. 2002, Stadler et al. 2002, Tareke et al. 2002). Such foods may contain greater than 10 000 μg acrylamide kg^{-1} (Ahn et al. 2002). Another significant avenue for exposure to acrylamide is cigarette smoke (Schumacher et al. 1977, Bergmark 1997). Tareke et al. (2002) found detectable concentrations of acrylamide in foods with a long shelf-life, indicating that acrylamide is relatively stable once formed in foods. In the production of TT, polymer doses of 15 to 40 g PAM m^3 of slurry (or 100 to 500 g PAM $tonne^{-1}$ solids) (M.D. MacKinnon, Syncrude Canada Ltd., personal communication, 2003), which corresponds to potential acrylamide concentrations of 5 to 10 μg acrylamide L^{-1} release water (calculated from acrylamide concentrations in PAM formulations; assuming polymer dosage of 150 g polymer $tonne^{-1}$ solids, 7% solids content (Semple et al. 2001)). Thus, continuous acrylamide exposure from food consumption or cigarette smoke may pose more of a chronic health risk to the population than the occasional exposure to low concentrations produced by PAM use. The two routes (food and cigarette smoke) are ingested directly, and so do not have the opportunity for degradation.

Acrylamide is water-soluble and does not readily adsorb to solids (Habermann 1991), so following deposition, acrylamide likely remains associated with the water phase in oil sands tailings. Results of Section 2.2.2.1.1 confirm this hypothesis, because when acrylamide was added directly to TT, blended thoroughly, and volumes of the TT analyzed immediately, the concentration corresponded to the expected concentration (from calculation). Furthermore, acrylamide did not adsorb to the plastic (Section 2.2.2.1.2) or glass bottles (Sections 2.2.2.2, 2.2.2.3, 2.2.2.4) used in any experiments.

The findings of Brown et al. (1980a) support this hypothesis, with little adsorption of acrylamide to montmorillonite or kaolinite clay fractions. The Athabasca oil sands contain kaolinite and illite as major clay fractions (Schramm et al. 2000), and so acrylamide adsorption to these fractions will be minor. Acrylamide has a low vapor pressure (Thomas 1964), and so acrylamide was not expected to partition into the headspace of microcosms. Therefore, acrylamide added to the microcosms in this study likely remained in the liquid phase, and any loss of acrylamide throughout this study was due to factors other than chemical adsorption to the microcosm serum bottles or solids present in the microcosms.

Although tailings release water is not destined for potable use, the potential exists for acrylamide present in pore water to migrate into vegetation planted in reclamation areas, and potentially be made available to grazing fauna (Kindzierski 2001). Acrylamide concentrations are low and bioaccumulation potential is small, so risk is expected to be minor. However, the relatively high dosages of PAM used as a flocculant in the oil sands tailings may still be an environmental concern because of its potential as a human carcinogen. As a result, issues regarding the stability of residual acrylamide in the tailings deposits must be addressed. Initially, conditions will be aerobic, because oxygen is introduced into the tailings by agitation during tailings transport and treatment. Once oxygen has been depleted as the TEA in TT, other processes requiring alternate TEAs such as sulfate and bicarbonate occur. Although no characterization of the microorganisms in TT has been completed to date, Holowenko et al. (2000) demonstrated the presence of active communities of SRB and methanogens in MFT. Similar communities are expected in TT because of the common origin of the tailings. Thus, laboratory examinations of biodegradability of acrylamide in this study included aerobic, sulfate-reducing, and methanogenic conditions.

Once oxygen has been depleted in the oil sands tailings, sulfate-reducing conditions will likely predominate. In the literature, there are no studies investigating acrylamide degradation by SRB; thus, this study was the first to investigate the potential of SRB to degrade acrylamide, as well as a potential degradation metabolite, acrylic acid. Acrylic acid is a well-known intermediate of acrylamide degradation under aerobic conditions (Nawaz et al. 1993, Zabaznaya et al. 1998, Bernet et al. 1987, Kumar and

Kumar 1998, Shanker et al. 1990), but no published reports exist regarding the fate of acrylic acid under sulfate-reducing conditions. Initial studies under sulfate-reducing conditions were promising, with acrylamide and acrylic removal demonstrated in cultures inoculated with MFT, TT, or mud as sources of microorganisms (Figure 3.8, Figure 3.9). However, later transfers designed to demonstrate substrate loss coupled to sulfate reduction were not successful, because little substrate or sulfate loss was detected (Figure 3.10). Nonetheless, acrylamide was biodegraded when it was added to sulfate-containing medium with MFT, TT, or mud (Table 3.5).

When sulfate has been depleted, methanogenic conditions predominate in oil sands tailings. Bicarbonate is present at approximately 1000 mg L^{-1} in oil sands tailings (Holowenko et al. 2002), and so an adequate supply of TEA exists for methanogenesis. Although methanogenesis has been observed in oil sands tailings, the effect of adding acrylamide was unknown. No reports of acrylamide degradation under methanogenic conditions have been made, so this study was the first to investigate the effect of acrylamide on methanogenesis.

Three possibilities exist for the effect of acrylamide on methanogenesis: (1) stimulation of methanogenesis, (2) no effect, or (3) inhibition of methanogenesis. All three possibilities were observed in this study. Methanogens are limited to simple compounds containing few carbon atoms as substrates (Zinder 1993). Although acrylamide contains only three carbon atoms, it likely is not used by methanogens. At higher concentrations (360 and 710 mg L^{-1}), acrylamide inhibited methanogenesis in a sewage-sludge consortium initially (Figure 3.15), but the inhibitory effects were temporary, as an acclimated consortium demonstrated stimulated methanogenesis in the presence of acrylamide (Figure 3.16). Lower acrylamide concentrations (7, 40, and 70 mg L^{-1}) had no effect on methanogenesis (Figure 3.15).

A reported value for a first-order degradation rate constant (k_1) for acrylamide in soil has been reported as 2.8×10^{-4} d^{-1} (U.S. EPA 2001). This corresponds to a half-life of 2500 d, which is considerably longer than any of the aerobic, anaerobic, or simulated field condition half-lives generated in this study (Table 3.1, Table 3.2, Table 3.5). The longest half-life calculated from the results of this study was 116 d (TT-inoculated microcosms under methanogenic conditions), which is approximately one-twentieth of

the literature value. Results in this study have given average rates of 0.27 d^{-1} and 0.32 d^{-1} under aerobic and anaerobic conditions, respectively, in the TT deposit (Table 3.2). At higher acrylamide concentrations, the rate does not differ greatly between aerobic and anaerobic conditions. Although temperatures in the winter slow degradation rates, once the temperature reaches 4°C, as in the spring, summer and autumn, acrylamide removal becomes rapid (Section 3.1.1.1.1). Thus, acrylamide likely will not persist under the conditions expected in the TT deposit.

4.2. PAM

High molecular weight PAMs are considered safe for uses such as drinking water treatment, food processing, as an ingredient in cosmetics (Lipp and Kozakiewicz 1991, European Chemicals Bureau 2002, Kindzierski 2001). However, concerns over the safety of PAM use have prompted studies into the degradability of high molecular weight polymers. The carbon backbone of PAM is considered quite resistant to microbial attack, but the nitrogen contained in its acrylamide subunits is considered to be a source of nitrogen for some microorganisms (Kay-Shoemake et al. 1998a, Grula et al. 1994). Thus, this study focused on determining the extent to which PAM could serve as a nitrogen source under aerobic, sulfate-reducing, and methanogenic conditions, as was discussed in Section 3.2. Acrylamide release from PAM was not believed to be thermodynamically possible, and so PAM as a carbon source was not investigated.

In an oil sands tailings environment, deamination of PAM, as well as residual acrylamide, may occur without a definite identification of the origin of the amidases involved. Extracellular amidases are ubiquitous in soil, and although a certain amount is inactivated by microbial degradation, some amount of free enzyme persists in soils (Skujiņš 1976). A similar situation may occur in oil sands tailings, with different members of the microbial community producing extracellular amidases. Amidase activity was detected in aerobic samples originating from process water used in the extraction of oil sand (Section 2.2.3.1), and so such amidases may accumulate over time. An amidase activity assay of the extruded pore water would provide verification of the presence of extracellular amidases in the oil sands tailings.

Once other terminal electron acceptors such as sulfate have been depleted, methanogenesis will occur in oil sands tailings. Methanogenesis has been observed in established tailings deposits such as MLSB for approximately 10 y, with a predicted methane production of 0.25 mL methane g^{-1} MFT (Holowenko et al. 2000). Although methanogenesis has not yet been observed in TT deposits, development of methanogenic conditions in MLSB took more than 10 y, so such conditions may develop eventually as the TT deposit matures. One important difference between the two environments is the presence of PAM in TT, and the PAM may impact methanogenesis.

Although MFT from the Mildred Lake site and TT from the Aurora site both originate from oil sands environments, several differences in the activity of the two inocula have been revealed through this study. Under sulfate-reducing conditions, sulfate loss was detected in MFT-inoculated enrichment cultures when acrylamide and acrylic acid, or acrylic acid alone, were added as carbon sources, whereas cultures containing TT did not demonstrate sulfate loss (Table 3.3). This suggests that the MFT may have developed a more numerous SRB community as compared to the more recently-formed TT deposits, but as the TT deposit ages, SRB may increase in numbers. No enumeration of SRB in TT was completed in this study, but SRB were active, as indicated by formation of black precipitate on iron nails in medium designed to detect SRB (Section 3.1.1.3). Although differences in processing, ore composition, or deposit age may account for these differences in activity, further work must be done to clarify the reasons for the differences. Future work enumerating the SRB over time in the TT deposit would be useful to determine whether temporal differences exist between MFT and TT SRB communities. As well, characterizing the SRB communities in MFT and TT using molecular methods such as PCR-RFLP may help clarify the differences in activity observed in this study.

Differences between the microbial communities in MFT and TT were also observed under methanogenic conditions. When supplied with benzoate as a carbon source, TT-inoculated microcosms demonstrated a much higher enhancement of methane production in a shorter timespan over viable cultures lacking PAM than did MFT-inoculated cultures (Section 3.2.2.1). This may be due to differences in the microbial

consortium present, and so characterizing the methanogenic communities using molecular methods may be useful.

One further use for molecular analysis of anaerobic communities used in this study is to characterize the sewage consortium capable of PAM-C, and compare the results to consortia in TT and MFT to determine which consortium members may be responsible for this activity.

4.3. Fulfillment of objectives

The objectives of this research were to predict the fate of residual acrylamide in the TT deposits, and determine the effect of PAM on the microbial community present in TT. The major findings of the laboratory studies are summarized below.

1. Acrylamide at low concentrations was readily biodegraded by an aerobic consortium enriched from TT.
2. Acrylamide at low concentrations was readily removed when added directly to TT under both oxic and anoxic conditions, and when the incubation temperature was above 4°C.
3. Acrylamide was removed from enrichment cultures under sulfate-reducing and methanogenic microcosms. Higher concentrations of acrylamide (360 and 710 mg L^{-1}) were initially inhibitory to methanogenic consortia, but acclimation restored activity, and acrylamide addition produced methane. Microcosms under "sulfate-reducing" conditions degraded acrylamide and acrylic acid in enrichment cultures, but sulfate loss coupled to substrate removal could not be demonstrated to verify that SRB were responsible for this activity.
4. PAM stimulated methanogenesis in oil sands tailings and sewage consortia when provided as a nitrogen source. However, no evidence that PAM could serve as a nitrogen source under sulfate-reducing conditions was observed. PAM served as a nitrogen source for garden

soil and oil sand extraction process water consortia under aerobic conditions.

4.4. Future work

Several interesting observations lead to opportunities for future work. The ability of sewage sludge-inoculated methanogenic microcosms to use PAM as a carbon source lead to several questions. First, which unique members of the consortium are responsible for this activity? None of the MFT-, TT-, or other sewage sludge-inoculated cultures in this study demonstrated any methanogenesis provided with PAM as sole carbon source. Molecular biology methods could be used to elucidate which members are unique to this culture, and could also be used to compare to the consortia present in other environmental samples. Second, which part of the PAM chain is being used as a carbon source? ^{14}C-labeled PAM could be used to identify which carbons are being used, which could aid in determining the route of degradation. In order to elucidate this pathway, more refined methods for PAM analysis must be developed.

Another area to be explored is the effect of physical degradation and photodegradation on PAM in TT deposits. Some shear will likely occur during deposition, which may reduce the molecular weight of PAM. Depending on the conformation of the deposition sites (wide, long pits vs. narrow deep pits), deposited TT may have large surface areas that are exposed to sunlight. Thus, the molecular weight of the polymer may be reduced, and as previously discussed, molecular weight reduction can lead to increased mineralization.

Methanogenesis was significantly enhanced by PAM when supplied as a sole nitrogen source. Amidases are likely responsible for liberating ammonium, but the source of the amidases is unknown. Further research should investigate the origin of theses amidases; particularly, are methanogens capable of amidase production? There are no literature reports of amidase production by methanogens, so this is one avenue of exploration.

The role of SRB in both the deamination of PAM and the degradation of acrylamide must be further clarified. This study failed to detect sulfate loss coupled to

acrylamide removal under the laboratory conditions tested. In the future, more work should be done to ensure that the medium formulation is optimal for SRB growth with acrylamide.

Acrylamide stimulated methanogenesis, with the production of an unidentified metabolite, but the route of acrylamide degradation was not verified. Although the metabolite is believed to be acrylic acid based on HPLC retention time, no verification of this identification was possible. Thus, further research should clarify the route of acrylamide biodegradation under both sulfate-reducing and methanogenic conditions.

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6. Appendix A

6.1. Measuring PAM-N content

To verify that microorganisms use nitrogen from PAM as their nitrogen source, the amount of nitrogen either remaining on or released from the polymer must be determined. Elevated ammonium concentrations in culture medium would provide evidence of release of the amide group of PAM, but no ammonium has been detected in culture medium, likely because of biological uptake. Thus, two methods of quantifying the amount of nitrogen remaining on the PAM were investigated, chemiluminescence and acid hydrolysis.

6.1.1. Chemiluminescence

To assay for organic nitrogen by chemiluminescence, a 500 mg PAM L^{-1} solution was prepared and diluted in sterile methanogenic medium to give standards ranging from 1 to 29 mg PAM-N L^{-1}. Volumes of 6 μL were injected in triplicate into an Antek 7000V elemental analyzer for nitrogen and sulfur (Antek Instruments Inc., Houston, TX) with a combustion chamber temperature of 1050°C. Chemiluminescence measures only organic nitrogen, as is found in PAM at a level of 11.7% (w/w). Data were gathered and manipulated using HP Chemstation software, version A.03.34.

The chemiluminescence method produced a linear standard curve when PAM was dissolved in water, as shown in Figure 6.1. However, when used to quantify PAM-N in cultures, results were not consistent, so this method was abandoned in favor of the acid hydrolysis method (Section 6.1.2)

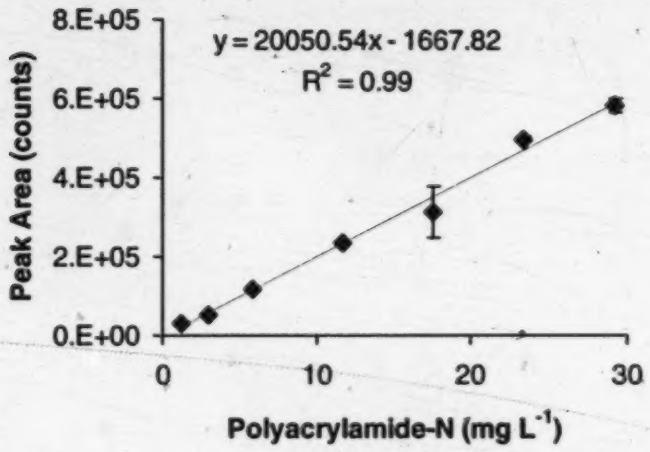

Figure 6.1. Calibration curve for PAM dissolved in water using the chemiluminescnce method. Error bars represent standard deviation of n=3.

6.1.2. Acid hydrolysis

Acid hydrolysis of PAM was performed according to the method of Challis and Challis (1970) as adapted by Kay-Shoemake et al. (1998b). PAM standards ranging from 4 mg L^{-1} to 500 mg L^{-1} were prepared in water, and 0.5 mL of 12 M HCl were added to 1.5 mL portions of PAM solutions. The standards were mixed briefly by vortexing, sealed with Thermowell™ aluminum sealing tape, and placed in boiling water for 30 min. When cool, the standards were neutralized with 10 M NaOH, and the amount of ammonium released was determined using the method described in Section 2.1.7. Verification was performed using a total of three PAM formulations.

Acid hydrolysis of PAM produced linear standard curves within the concentration range of 4 mg L^{-1} to 500 mg L^{-1}, as shown in Figure 6.2.

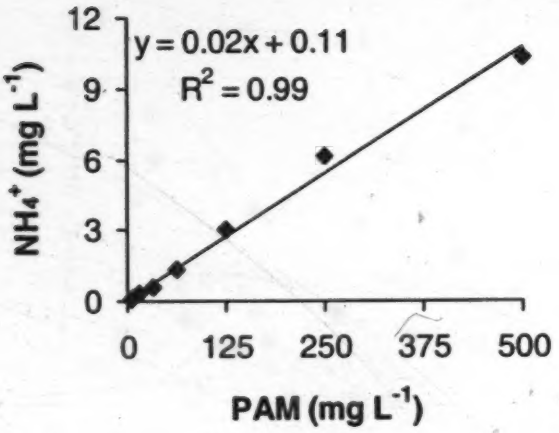

Figure 6.2. Ammonium release from anionic PAM treated by acid hydrolysis.

One observation noted during the initial experiment was that although the amount of ammonium released was linear, the amount released was only 15.3 ± 2.4 % of the total nitrogen present in the PAM, as shown in Table 6.1. One possible reason is the formation of nitrogen-containing imide ring structures when PAM is treated with strong acids (Thomas 1964), thereby limiting the amount of nitrogen released as ammonium. This may be problematic when acid hydrolysis is used to monitor PAM deamination in cultures, because the proportion of nitrogen released as ammonium may not be consistent with PAM concentration. Although this proportion remained relatively constant in repeated experiments, verification was necessary, so two other commercial PAM formulations (Alcoflood 1235 and Magnafloc 336) were tested.

Two other formulations of PAM were tested by acid hydrolysis to determine if the amount of PAM-N recovered was constant (Figure 6.3). Statistical analysis revealed that the only significant difference between the formulations was between Alcoflood 1235 and Magnafloc LT27AG ($p<0.05$).

Table 6.1. Nitrogen released as ammonium by acid hydrolysis from Magnafloc® LT27AG PAM.

PAM (mg L^{-1})	Expected N in solution (mg L^{-1})	Ammonium detected after hydrolysis (mg L^{-1})	N recovered as ammonium (% of total N added)
500	59	10	13.7
250	30	6.2	16.3
125	15	3.0	15.8
63	7.3	1.3	14.0
31	3.7	0.62	13.0
16	1.8	0.36	15.1
8	0.9	0.16	13.7
4	0.5	0.12	20.5

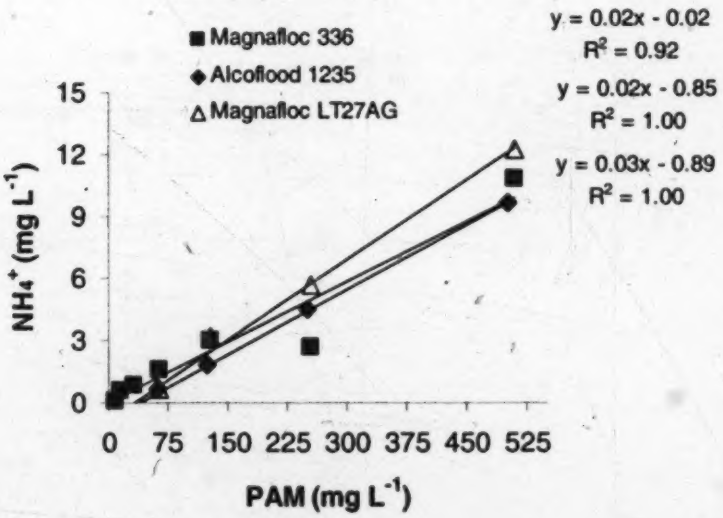

Figure 6.3. Comparison of ammonium recovery of three PAM formulations treated by acid hydrolysis. Trendline equations are listed in legend order.

When the amount of nitrogen recovered was expressed as a percentage of the total PAM-N added, which differs among formulations (see Table 2.1), slightly different results were observed (Table 6.2). While the amount of nitrogen recovered from Alcoflood 1235 samples was not significantly different from Magnafloc LT27AG, there was a significant difference between Magnafloc 336 and Magnafloc LT27AG. However, all three formations had mean recoveries between 13 and 18%, which had been previously observed in experiments with Magnafloc LT27AG (data not shown).

Table 6.2. Nitrogen recovery following acid hydrolysis from three commercial PAM formulations.

PAM ($mg\ L^{-1}$)	N recovered (% of total N added)		
	Magnafloc 336	Alcoflood 1235	Magnafloc LT27AG
500	13.4	16.0	15.6
250	ND[1]	14.9	13.6
125	15.5	16.1	14.7
63	14.3	17.0	11.3
31	16.1	22.6	10.5
16	24.7	24.0	12.2
8	12.1	13.2	11.1
4	14.9	ND	16.6
Mean N recovery (%)	15.9[2]	17.7	13.2

[1] ND – not done

[2] indicates recovery significantly different from LT27AG mean (p=0.06).

6.1.3. Evaluation of interferences in acid hydrolysis of PAM

The acid hydrolysis method was tested to ensure that ammonium detection resulting from PAM hydrolysis would not be affected by the presence of other N-containing materials such as proteins, DNA, or whole cells. For all experiments, a series of PAM standards (Magnafloc® LT27AG, Ciba® Specialty Chemicals, Mississauga, ON)

ranging from 4.0 x 10^{-4} to 0.051% (w/v) (yielding 4.3 to 0.035 mM PAM-N) was prepared in sterile water. To test for the effect of protein, a sufficient volume of a 2 mg BSA mL^{-1} stock solution was added to each standard to achieve a final concentration of 10 μg protein mL^{-1}. To test the effect of bacterial DNA, 2μL of purified *Pseudomonas fluorescens* LP6a DNA (obtained from Stephanie Cheng, Department of Biological Sciences, University of Alberta) were added to 1.5 mL of each standard. To test the effect of whole cells, 100 μL of a 0.59 OD_{600} *P. fluorescens* LP6a cell suspension (courtesy of Kathy Semple, Department of Biological Sciences, University of Alberta) were added to 1.5 mL of each standard. Standards were mixed well by vortexing, and were subjected to acid hydrolysis and ammonium quantification.

The results of these analyses are shown in Figure 6.4. The calibration lines were compared by testing whether there were statistically significant differences between their slopes or y-intercepts in the presence or absence of biological materials. The addition of protein had no statistically significant effect on recovery of PAM-N ($p<0.05$ for slope and intercept) (Figure 6.4a). Both whole cells of *P. fluorescens* and DNA significantly increased the amount of nitrogen recovered ($p>0.05$ for intercept) (Figure 6.4b), but only DNA significantly affected the slope of the line (Figure 6.4b).

Although the acid hydrolysis method produced a linear standard curve when standards were prepared in water (Figure 6.2), the method was not useful for detecting nitrogen loss from PAM in culture medium, and so was not used to quantify PAM-N loss in cultures.

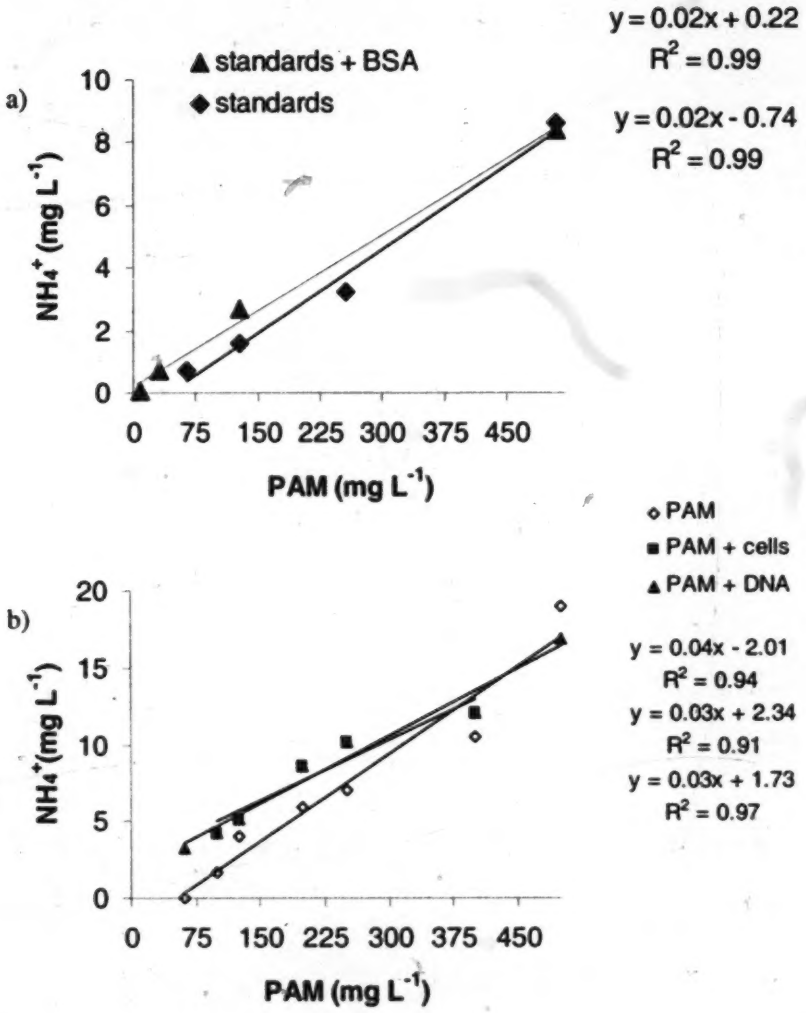

Figure 6.4. Effect of nitrogenous components of microbial cells on ammonium-N release from PAM following acid hydrolysis; a) protein, b) whole cells and DNA. Trendline equations are listed in legend order.

6.2. PAM as a nitrogen source in glucose-containing cultures under methanogenic conditions

To enrich for a wider variety of anaerobic microorganisms in methanogenic consortia, glucose was used as a carbon source following an initial enrichment on acetate. All sources of environmental microorganisms tested removed glucose more rapidly compared to acetate and benzoate, but with dramatically lower methane production. MFT-inoculated cultures both containing and lacking PAM removed ~ 94% of the glucose by d 136, as shown in Figure 6.5a. Methane production was considerable lower than in acetate-containing cultures, with average maximum production of 7% and 5% in cultures containing PAM and with no additional nitrogen respectively (Figure 6.5b).

In cultures inoculated with TT, glucose was completely removed in cultures supplemented with PAM by d 28 (Figure 6.6a), which was also when methane production reached its maximum of 2.9% (Figure 6.6b). Glucose was also removed from cultures that had no added PAM, but 12% remained on d 35. Methane production was only a third of that produced by PAM-containing cultures, with a maximum value of only 1%.

Glucose removal by sewage sludge-containing microcosms was very rapid, with 86% of the added glucose removed by d 7 by all cultures, shown in Figure 6.7a. No differences were observed between cultures containing and lacking PAM, as was the case with methane production (Figure 6.7b). The maximum methane production was quite low compared to that of acetate-containing cultures, with average values of 8% and 9% for cultures with no added nitrogen and cultures containing PAM, respectively. Although cultures inoculated with TT did display enhanced methane production when provided with PAM as a nitrogen source, neither MFT- nor sewage-inoculated cultures showed the same trend. Neither one demonstrated significantly different methane production when supplemented with PAM.

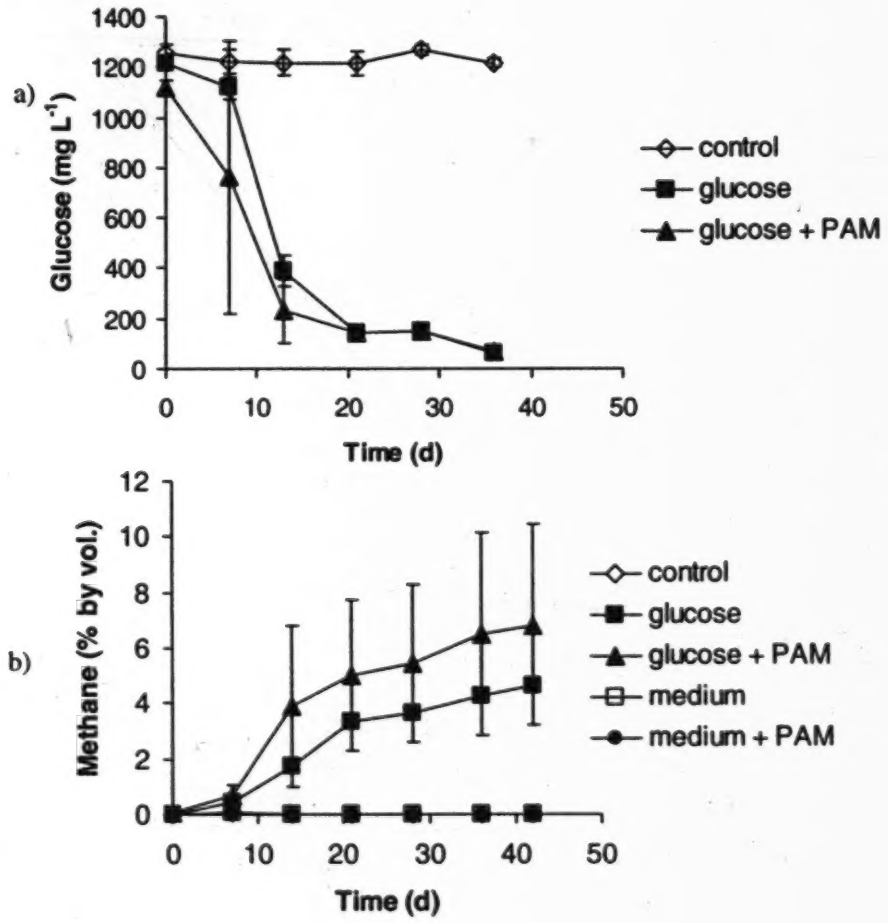

Figure 6.5. Activity of microcosms containing MFT with glucose as carbon source and PAM as nitrogen source. a) glucose removal, b) methane production. Error bars represent standard deviation of n=3.

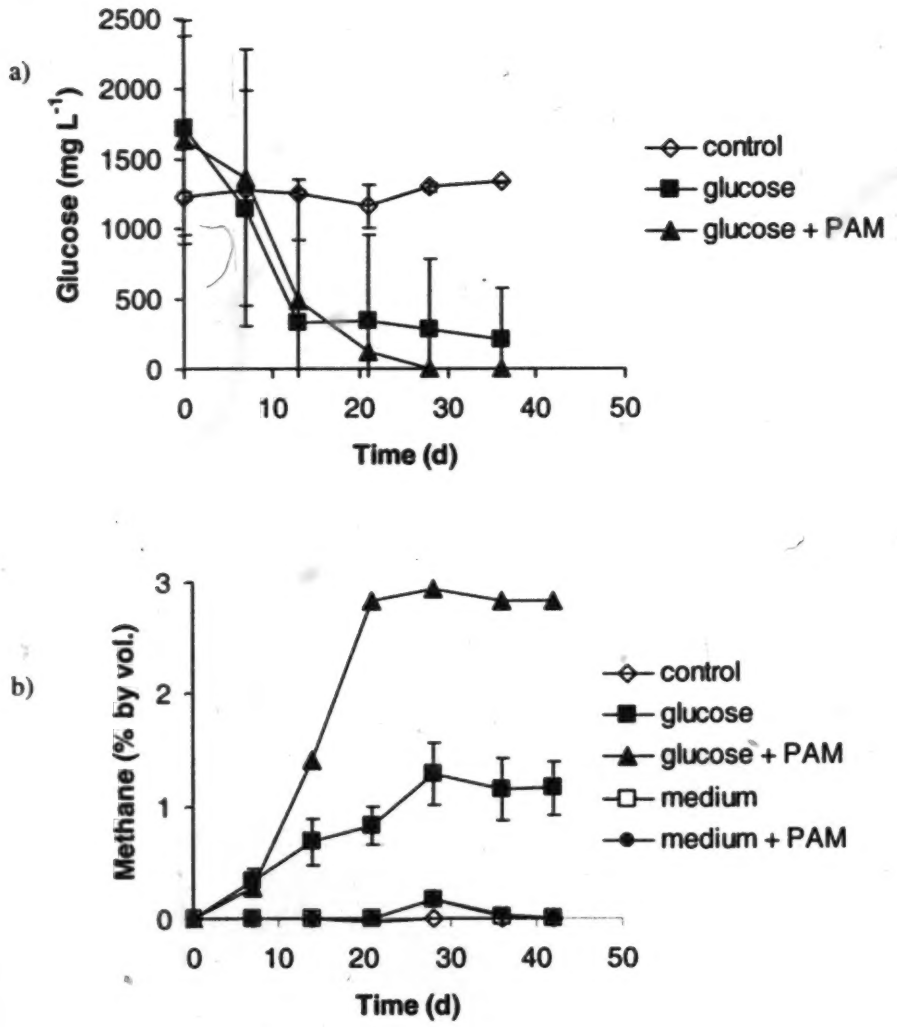

Figure 6.6. Activity of microcosms containing TT with glucose as carbon source and PAM as nitrogen source. a) glucose removal, b) methane production. Error bars represent standard deviation of n=3.

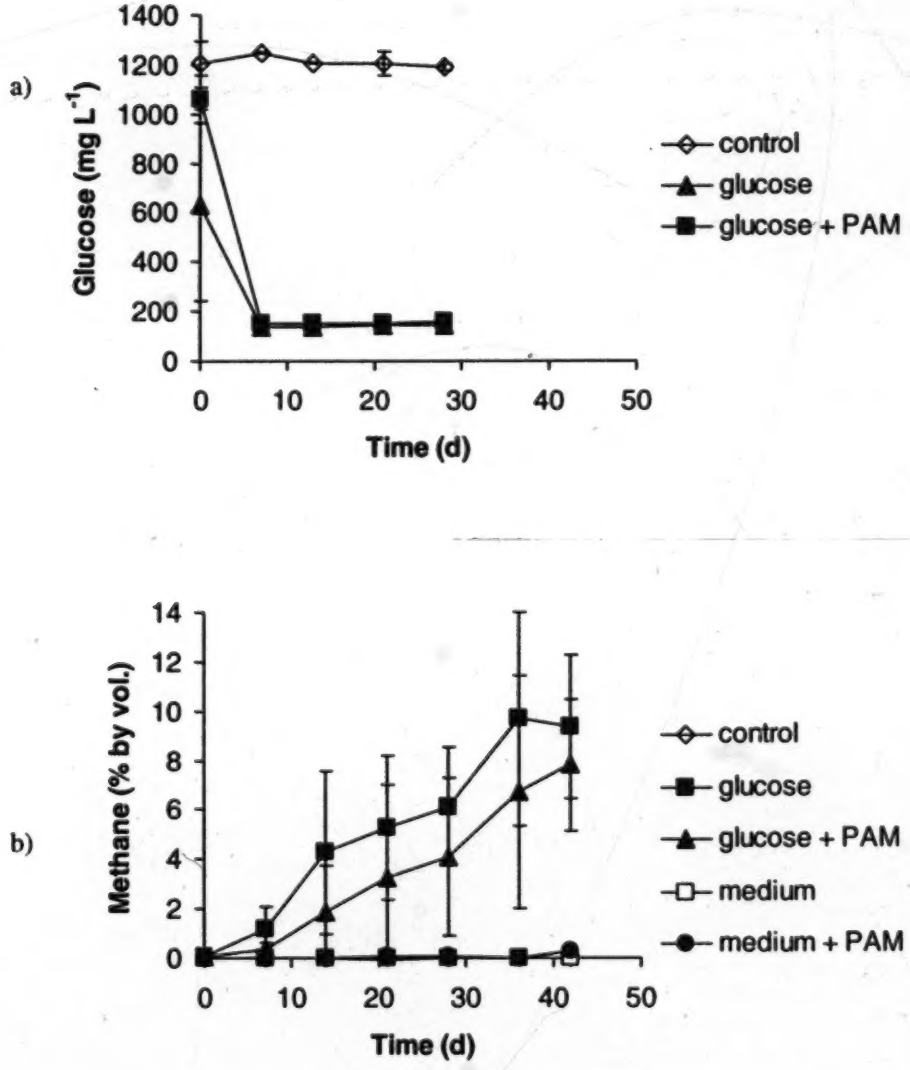

Figure 6.7. Activity of microcosms inoculated with sewage with glucose as carbon source and PAM as nitrogen source. a) glucose removal, b) methane production. Error bars represent standard deviation of n=3.

One puzzling observation is that the methane yield in all the cultures was considerably lower than is expected. Approximately 30% (by vol.) methane is expected

from an initial concentration of 1000 mg glucose L^{-1} according to Buswell's equation (Suflita et al. 1997). The reason for this anomaly is unknown.

Despite the low methane yield, PAM addition to microcosms containing microbes enriched from TT increased methane production (Figure 6.6), suggesting that PAM was serving as a nitrogen source for these consortia.